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EPA METHOD STUDY 15,
METHOD 605--BENZIDINES

by

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16. ABSTRACT

Seventeen laboratories participated in an interlaboratory study conducted to provide precision and accuracy statements for the proposed EPA Method 605 for measuring concentrations of the Category 7 chemicals benzidine and 3,3'dichlorobenzidine (DCB) in municipal and industrial aqueous discharges. Method 605 involves extraction of benzidine and DCB from the aqueous sample at pH 7-8 with chloroform. The extract is then back extracted into acid, re-extracted into chloroform at neutral pH, and concentrated The benzidines are determined in the final extract using high performance liquid chromatography (HPLC) with electrochemical detection.

The study design was based on Youden's plan for collaborative tests of analytical methods. The resulting data were statistically analyzed using the computer program "Interlaboratory Method Validation Study" (IMVS).

Mean recoveries of benzidine and DCB ranged from 48-70 percent. Overall precision ranged from 38-69 percent and single-analyst precision ranged from 24-40 percent.

A statistically significant effect due to water type was established for DCB in the case of one wastewater. However, review of the precision statistics indicated the effect was of no practical importance. There were no other discernible differences due to water types among mean recoveries, overall precisions or single-analyst precision.

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory (EMSL)-Cincinnati conducts research to:

- Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.

- Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microorganisms in water.

- Determine the responses of aquatic organisms to water quality.

- Support an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring waste and wastewater.

This publication, EPA Method Study 15, Method 605 (Benzidines) reports the results of the interlaboratory method study conducted by EMSL-Cincinnati, for Benzidine (92-87-5)* and 3,3'-Dichlorobenzidine (91-94-1)*.

Federal agencies, states, municipalities, universities, private laboratories, and industry should find this evaluative study of assistance in monitoring and controlling pollution in the environment.

Robert L. Booth
Director, EMSL-Cincinnati

*CAS Registry Number

ABSTRACT

The U.S. Environmental Protection Agency (USEPA) sponsored an interlaboratory study in which 17 laboratories participated to provide precision and accuracy statements for the proposed EPA Method 605 for measuring concentrations of the Category 7 chemicals benzidine and 3,3'-dichlorobenzidine (DCB) in municipal and industrial aqueous discharges. Method 605 involves extraction of benzidine and DCB from the aqueous sample at pH 7-8 with chloroform. The extract is then back extracted into acid, re-extracted into chloroform at neutral pH, and concentrated. The benzidines are determined in the final extract using high performance liquid chromatography (HPLC) with electrochemical detection. If interference in the measurement of benzidine is encountered, the method provides additional detector settings to increase the selectivity of the analytical system.

The study design was based on Youden's non-replicate plan for collaborative tests of analytical methods. Three Youden pair samples of the test compounds were spiked into six types of test waters and then analyzed. The test waters were distilled water, tap water, a surface water, and three different industrial wastewater effluents. The resulting data were statistically analyzed using the computer program entitled "Interlaboratory Method Validation Study" (IMVS).

Estimated mean recoveries of benzidine and DCB based upon the slope of the regression equations ranged from 48-70 percent. Overall precision ranged from 38-69 percent and single-analyst precision ranged from 24-40 percent.

A statistically significant effect due to water type was established for DCB in the case of one wastewater. However, review of the precision statistics indicated the effect was of no practical importance. There were no other discernible differences due to water types among mean recoveries, overall precisions or single-analyst precision.

The study was conducted at the request of the USEPA's Quality Assurance Branch, EMSL - Cincinnati, Ohio under EPA Contract No. 68-03-2624. This report covers a period from September 13, 1978 to October 31, 1983, and work was completed as of December 31, 1982.

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Gratitude is also expressed to various industries for their interest in the study and willingness to provide samples of appropriate wastewater effluents without which the program's objectives could not have been realized. Lastly, we thank Mr. Edward L. Berg and Mr. Robert Graves, EPA Project Officers, whose guidance and intense interest in the study contributed to the successful completion of a difficult and sometimes frustrating task.

SECTION 1

INTRODUCTION

USEPA first promulgated guidelines (1) establishing test procedures for the analysis of pollutants in 1973, following the passage of the Federal Water Pollution Control Act in 1972 by Congress. Pursuant to the amendment and publication of these guidelines, USEPA entered into a Settlement Agreement--the Consent Decree--which required the study and, if necessary, regulation of 65 "priority" pollutants and classes of pollutants of known or suspected toxicity to the biota. Subsequently, Congress passed the Clean Water Act of 1977(2), mandating the control of toxic pollutants discharged into ambient waters by industry.

To facilitate the implementation of the Clean Water Act, USEPA selected 113 organic and 16 inorganic toxic pollutants for initial study. The organic pollutants were divided into 12 categories based on their chemical structure. Analytical methods were developed by USEPA for these 12 categories through in-house and contracted research.

This report describes the interlaboratory study of Method 605 which is proposed for the Category 7 chemicals: benzidine and DCB. The primary objective of the study was to characterize the behavior of Method 605 in terms of: 1) accuracy; 2) overall precision; 3) single-analyst precision; and 4) effect of water type on accuracy and precision. The study was conducted with the cooperation of 17 participating laboratories under auspices of EMSL - Cincinnati.

Each of the laboratories was supplied with six samples of the Category 7 chemicals as solutions in methanol packaged in flame-sealed glass ampules. One-mL aliquots of the solutions were added to six types of test waters according to instructions. Water samples were then analyzed with and without the increment, and the added level of pollutant determined by

difference. Concentrations were reported in µg/L with only one analytical value reported per analyte per each spiked solution.

The data were collected from the 17 laboratories according to Youden's(3) collaborative testing design. Formal statistical techniques compatible with the Youden design were used to identify outliers, estimate the method's accuracy and precision and test for the effect of water type. The formal statistical analyses were carried out using USEPA's IMVS computer program(4). The information obtained from the statistical analyses was summarized and reduced to a descriptive form for the purpose of interpretation and presentation.

Method 605 was developed in the Battelle-Columbus Laboratories (BCL) under a contract with the Physical and Chemical Methods Branch, EMSL - Cincinnati and is presented in Appendix A.

SECTION 2

SUMMARY

The interlaboratory study of an analytical process such as Method 605, involves characterizing the accuracy and precision of the method over a wide range of working conditions. Accuracy is directly related to mean recovery and precision is related to both the overall standard deviation and the single-analyst standard deviation of the method.

A range of analytical conditions was introduced into the study by varying the concentration levels of the analytes spiked into the sample test water and by changing the type of test water. For each analyte, three concentration levels (low, medium, and high) were used with each of six different test waters (distilled water, tap water, surface water, and three industrial wastewater effluents).

For purposes of summarizing the accuracy of Method 605, the following equation was determined for each effluent:

$$X = a + b \cdot C$$

where X denotes the mean recovery and C the concentration level of the analyte. The precision was summarized for each effluent by the equations

$$S = d + e \cdot X \text{ and } SR = f + g \cdot X$$

where S denotes the overall standard deviation and SR the single-analyst standard deviation.

The data reported by the 17 participating laboratories were used to estimate the coefficients a, b, d, e, f, and g for these equations for each effluent. These equations were calculated for each effluent used in this study, and the results are summarized in Table 1.

Based upon the slopes of the equations at concentrations above 20 µg/L, percentage recoveries for benzidine and DCB ranged from 52-70 and 48-66, respectively, for all water types. The overall percent relative standard deviation for benzidine and DCB ranged from 40-69 and 38-58 percent, respectively, for all water types. The single-analyst percent relative standard deviation for benzidine and DCB ranged from 24-40 and 26-40, respectively, for all water types.

In addition to summarizing the accuracy and precision of Method 605, the following observations were made based on the data:

- The method has a pronounced negative bias for both compounds at all concentration levels in all water types.
- In general, there are no discernible differences in accuracy or precision due to water types with the exception that wastewater C-74 yielded statistically significant lower recoveries than distilled water for DCB. However, it was determined on the basis of overall and single-analyst precision data that the observed effect is of no practical importance.
- The maximum recovery of DCB from water is approximately 75 percent as a result of its limited extraction efficiency into 1 M sulfuric acid from chloroform.
- Benzidine and DCB are readily oxidized and sources of oxidant must be carefully avoided.

- The basic character of the compounds requires careful pH control throughout the workup procedure.
- Determination of benzidine at 0.6 Volts vs. Ag/AgCl is very useful in reducing interference problems when analyzing actual wastewaters. Even lower potentials may be useful with certain electrochemical detectors. The lowest potential demonstrating a good response for benzidine should be employed for complex wastewaters.
- The number of outliers for each data set as determined by the ranking and individual outlier test ranged from one to nine, and averaged about four outliers per data set. Twenty-four percent of the data for the two compounds were rejected.

TABLE 1. REGRESSION EQUATIONS FOR ACCURACY AND PRECISION OF METHOD 605 BY COMPOUND AND WATER TYPE

WATER TYPE	BENZIDINE	3,3-DICHLOROBENZIDINE
APPLICABLE CONC. RANGE	(1.00 - 55.00 µg/L)	(1.00 - 70.00 µg/L)
DISTILLED WATER		
SINGLE-ANALYST PRECISION	SR = 0.28X + 0.19	SR = 0.39X - 0.05
OVERALL PRECISION	S = 0.40X + 0.18	S = 0.38X + 0.02
ACCURACY	X = 0.70C + 0.06	X = 0.66C + 0.23
TAP WATER		
SINGLE-ANALYST PRECISION	SR = 0.32X + 0.42	SR = 0.29X + 0.06
OVERALL PRECISION	S = 0.50X + 0.21	S = 0.38X + 0.16
ACCURACY	X = 0.64C + 0.32	X = 0.64C + 0.12
SURFACE WATER		
SINGLE-ANALYST PRECISION	SR = 0.30X + 0.09	SR = 0.26X + 0.12
OVERALL PRECISION	S = 0.56X + 0.06	S = 0.40X + 0.10
ACCURACY	X = 0.57C + 0.01	X = 0.59C + 0.14
WASTEWATER (C-74)		
SINGLE-ANALYST PRECISION	SR = 0.40X + 0.04	SR = 0.27X + 0.28
OVERALL PRECISION	S = 0.69X - 0.13	S = 0.56X + 0.13
ACCURACY	X = 0.55C + 0.10	X = 0.48C + 0.03
WASTEWATER (C-75)		
SINGLE-ANALYST PRECISION	SR = 0.34X - 0.09	SR = 0.26X + 0.26
OVERALL PRECISION	S = 0.60X - 0.07	S = 0.58X + 0.09
ACCURACY	X = 0.62C - 0.18	X = 0.62C + 0.19
WASTEWATER (C-76)		
SINGLE-ANALYST PRECISION	SR = 0.24X + 0.10	SR = 0.40X + 0.05
OVERALL PRECISION	S = 0.64X - 0.01	S = 0.50X + 0.10
ACCURACY	X = 0.52C - 0.02	X = 0.53C + 0.08

X = MEAN RECOVERY
C = TRUE VALUE FOR THE CONCENTRATION

SECTION 3

DESCRIPTION OF STUDY

SELECTION OF PARTICIPATING LABORATORIES

Potential participants identified from a list of 100 laboratories provided by USEPA were informed of the impending study. Forty-one laboratories responded to the request for technical and cost proposals. Proposals were carefully reviewed and then ranked on the basis of technical experience and facilities. The laboratories selected for awards represented a mix of those showing average to above average analytical capabilities and are listed below:

Analytical Development Company
1875 Willow Park Way
P.O. Box 744
Monument, CO 80132

Arthur D. Little, Inc.
Acorn Park
Cambridge, MA 02140

Environmental Research Group, Inc.
117 N. First
Ann Arbor, MI 48103

Environmental Science and Engineering
P.O. Box 13454
Gainesville, FL 32604

Gulf South Research Institute
P.O. Box 26518
New Orleans, LA 70186

Mead Technology Laboratories
P.O. Box 12652
Research Triangle Park, NC
27709

Monsanto Research Corporation
P.O. Box 8, Sta. B
Dayton, OH 45407

Radian Corporation
8500 Shoal Creek Blvd.
P.O. Box 9948
Austin, TX 78766

Raltech Scientific Services, Inc.
P.O. Box 7545
Madison, WI 53707

Science Applications, Inc.
1200 Prospect Street
P.O. Box 2351
La Jolla, CA 92038

IIT Research Institute
10 West 35 Street
Chicago, IL 60616

Syracuse Research Laboratory
Merrill Lane
Syracuse, NY 13210

Southwest Research Institute
8500 Culebra Road
P.O. Drawer 28510
San Antonia, TX 78284

Systems, Science, and Software
3398 Carmel Mountain Road
San Diego, CA 02121

Southern Research Institute
2000 Ninth Avenue, S.
Birmingham, AL 35205

U.S. Environmental Protection
Agency
EMSL
26 W. St. Clair St.
Cincinnati, OH 45268

West Coast Technical Service
17605 Fabrica Way, Suite D
Cerritos, CA 90701

TEST DESIGN

The test design was patterned after Youden's original non-replicate plan(3) for collaborative evaluation of precision and accuracy for analytical methods. According to Youden's design, samples are analyzed in pairs, with each sample of a pair having a slightly different concentration of the constituent of interest. The analyst is directed to do a single analysis and report one value for each sample, as if for a normal routine sample.

Prior to the start of the formal interlaboratory study, a preliminary study was conducted to enable the participants to gain experience with the analytical and sample handling procedures. Each analyst analyzed one trial Youden pair sample and then attended a prestudy conference at USEPA in Cincinnati, Ohio, to discuss the findings, the method, and the factors that could affect the quality of data. The major discussions concerned cleanup of solvent extracts, HPLC conditions, and problems with the electrochemical detector. A number of recommendations were offered for improving method performance. Modifications to the method approved by USEPA are summarized in Appendix B.

To begin the formal interlaboratory study, test samples were prepared as liquid concentrates in sealed glass ampules and presented to the analyst with complete instructions. The analyst was required to add an aliquot of

each concentrate to a volume of distilled water and to a volume of each of the five water types described below. The analysis in distilled water was used to evaluate the analyst's ability to use the method on a sample free of interferences. Analyses in other water types were intended to reveal effects of various chemical matrices on the method's performance. Major elements of the test design were:

1. Three Youden pairs of samples were analyzed for each parameter and the concentrations within each pair deviated at least ±5-percent but no more than ±20-percent from the mean of the pair. The three Youden pairs were spread over a usable and realistic range, the lowest pair being slightly above the minimum detection limit, and within the linear range of the method.

2. Samples of approximately 2 mL were supplied as liquid concentrates in methanol contained in 10-mL glass ampules. One-half mL aliquots of the concentrates were added to the test waters according to the instructions. Water samples were analyzed with and without added increment and the added level determined by difference. Concentrations were reported in terms of µg/L with only one value reported for each sample.

3. Analyses were conducted in six types of water:

   a. Distilled water
   b. A municipal drinking water
   c. A surface water vulnerable to synthetic chemical contamination, as from a river
   d. Three industrial wastewaters from industries that normally would be regulated for the priority pollutants under study.

4. The participating laboratories were provided with the method of analysis, chemical standards for calibration, and samples of the three industrial wastewaters. Each participating laboratory was responsible for securing samples of test waters, a, b, and c.

SECTION 4

EXPERIMENTAL PROCEDURES

PREPARATION OF YOUDEN PAIR CONCENTRATES

Stock solutions of the Category 7 compounds were prepared by dissolving accurately weighed amounts of the chemicals in methanol. The sample concentrates were then prepared by diluting measured amounts of the stock solutions with methanol to 1 L. As noted, the concentration of a given compound in each Youden pair was adjusted so that it differed by at least ± 5 percent, but was no greater than ± 20 percent from the mean of the pair.

The concentrations of the diluted solutions were confirmed by liquid chromatographic analysis. Chromatographic conditions were the same as to those described in Method 605. Sample concentrates of approximately 2 mL of the diluted solutions were prepared in 10-mL glass ampules and flame-sealed. The concentrations, µg/mL, of the Category 7 compounds in the ampules are given in Table 2.

TABLE 2. CALCULATED CONCENTRATIONS OF CATEGORY 7 COMPOUNDS IN AMPULES, µg/mL

Compound	Ampule Number					
	1	2	3	4	5	6
Benzidine	12.0	55.0	1.0	40.0	8.0	1.4
DCB	15.0	70.0	1.0	55.0	11.0	1.4

STABILITY OF YOUDEN PAIR CONCENTRATES

The stability of the Youden pairs of Category 7 compounds in the ampules was studied over a 90-day period. Triplicate samples of each Youden pair concentrate were selected at random and analyzed at 0, 45, and 90 days. The stability of the samples was considered satisfactory. At the end of the 90-day period, recoveries of the compounds varied between 96 and 112 percent at each concentration level.

COLLECTION OF INDUSTRIAL WASTEWATER EFFLUENTS

Chemical industries producing Category 7 compounds or utilizing them in chemical processes were identified from Synthetic Organic Chemicals - U.S. Production and Sales 1974-77, U.S. International Trade Commission, and the 1979 Directory of Chemical Producers in the United States.

Since Method 605 may eventually be used by industries seeking permits issued by the National Pollutant Discharge Elimination System under the Clean Water Act, plants using good technology in their wastewater treatment facilities were selected for sampling. Attempts were made to obtain wastewater samples from both users and producers of the two category compounds. The appropriateness of a particular company's wastewater was first assessed by discussions with technical personnel of that company. If on the basis of these discussions, it appeared that the plant's effluent might be suitable for the program, a 1-gallon sample of the effluent was sent to BCL for a preliminary analysis. The effluent was judged to be suitable for the method study if the analyses showed no major interferences or high background of the two analytes that might nullify analytical data obtained for the lowest Youden pair concentrates. Approximately 40 gallons of acceptable wastewater effluents were collected in stainless steel drums at the plant sites by plant or BCL personnel and delivered to BCL by truck. Samples of wastewater were analyzed at BCL before and after spiking with benzidine and DCB. Prior to spiking and analysis, samples were treated with 35 mg/L of sodium thiosulfate to destroy residual oxidants and adjusted to pH 2.0 with 1 M sulfuric acid. If the results of these analyses were satisfactory, 500-mL samples of the effluent were treated in a similar

manner and prepared for shipment to the participating laboratories. The contents of the drums were stirred vigorously during the bottling operation to ensure sample homogeneity. Samples were identified by coded labels as C-74, C-75, or C-76. The three industrial wastewater effluents selected for the Category 7 method study were obtained from a manufacturer of pigments utilizing benzidine based dyes, a manufacturer of pigment inks, and a chemical manufacturer of aniline. The latter source was chosen because of the chemical potential of benzidine formation from aniline through oxidative coupling of two molecules of aniline.(5) A brief description of wastewater treatments used at these plants are given below. The results of BCL's analyses of the wastewater samples are given in Table 3.

Wastewater C-74 (SIC Code #2869*)--Effluent from a plant producing nitroaromatics and aromatic amines including aniline. Wastewater treatment includes biological treatment in an activated sludge plant followed by tertiary carbon treatment and then discharge into a river.

Wastewater C-75 (SIC Code #2869*)--Effluent from a plant producing dichlorobenzidine-based pigments. The wastewater stream is filtered, treated with a 4-8 percent solution of sodium hypochlorite, and then discharged into the municipal sewer.

Wastewater C-76 (SIC Code #2865*)--The wastewater stream is from a plant producing dichlorobenzidine-based pigment inks. The process water is adjusted to pH 6-10 with acid or alkali as required and sent to settling ponds. Effluent from the ponds is discharged into the municipal sewer.

---

* Standard Industrial Classification Code Number.

TABLE 3. BACKGROUND ANALYSES OF WASTEWATER SAMPLES FOR CATEGORY 7 COMPOUNDS, µg/L

Compound	Sample C-74	C-75	C-76
Benzidine	< 0.1	< 0.1	< 0.1
DCB	< 0.1	< 0.1	< 0.1

DISTRIBUTION OF SAMPLES

Each laboratory was provided with the following materials:

(1) Six Youden pair ampules of each of three concentration levels, for a total of 36 ampules for preparation of samples;
(2) Seven, 500-mL samples of three types of industrial wastewater effluents yielding a total of 21 samples;
(3) Standards of benzidine and DCB;
(4) A copy of Method 605;
(5) A form for reporting data; and
(6) A questionnaire requesting description of difficulties encountered with the analytical method and suggestions for improvement of the method.

The laboratories were instructed to add a measured quantity of the contents of the ampules to test waters by the following procedure:

> Place a magnetic stirbar in the sample bottle, add 0.5 mL of the solution in the ampule, cap and stir 15 minutes using a magnetic stirrer. Transfer the contents of the bottle to a 1-liter separatory funnel and proceed with the analysis described in Method 605, Benzidines. In calculating results, assume the volume of the sample to be 500-mL.

TREATMENT OF DATA

The study's objective was met through the use of statistical analysis techniques designed to extract and summarize the relevant information about accuracy and precision from the data reported by the participating laboratories. The statistical techniques employed in the data reduction process are similar to ASTM techniques.

The algorithms required to perform the statistical analyses have been integrated into a system of computer programs referred to as IMVS. The analyses performed by IMVS include tests for the rejection of outliers (laboratories and individual data points), summary statistics by concentration level for mean recovery (accuracy), overall and single-analyst standard deviation (precision), determination of the linear relationship between mean recovery and concentration level, determination of the linear relationship between the precision statistics and mean recovery, and a test for the effect of water type on accuracy and precision.

A detailed description of each of the statistical analysis procedures is presented below.

REJECTION OF OUTLIERS

Since the objective of the statistical analyses is to summarize the general behavior of the method in terms of accuracy and precision, it is important to identify data points which are inconsistent with the majority of the data. These data points should be deleted from the analysis because they can lead to values of summary statistics which are not representative of the general behavior of the method. However, some erratic behavior in the data may be directly related to some facet of the method under the study. Therefore, inconsistent data points should not be removed indiscriminately, and any points that are removed should be clearly identified since further investigation of the analytical conditions related to the outliers might be of value. In some cases the data points which are deleted may actually be closer to the true value than the retained data. This is consistent since outliers are judged relative to the average value of the data and not with respect to the true value. Data rejected as

outliers for this study as a result of any of the following tests for outliers have been identified by the symbol "*" in the raw data tables.

Youden's Laboratory Ranking Procedure

In some cases, the analytical values reported by a specific laboratory are so consistently high or low that a large systematic error may be attributed to that laboratory. These data are not representative of the method and should be rejected. Youden's(3) ranking test for outlying laboratories was applied separately to data from each of the waters used in this study. Since six water types were used in this study, the laboratory ranking procedure was applied to these six different subsets of the data. Each laboratory ranking test was performed at the 5 percent level of significance.

The Youden laboratory ranking procedure requires a complete set of data from every laboratory within a given water type. Missing data from laboratory "i" for water type "j" were replaced by the following procedure. Letting X_{ijk} denote the reported measurement from laboratory "i" for water type "j" and concentration level C_k, it is assumed that

$$X_{ijk} = \beta_j \cdot C_k^{\gamma_j} \cdot L_i \cdot \varepsilon_{ijk}$$

where β_j and γ_j are fixed parameters which determine the effect of water type "j", L_i is the systematic error due to laboratory "i" and ε_{ijk} is the random within laboratory error. Taking natural logarithms, it follows that

$$\ell n\, X_{ijk} = \ell n\, \beta_j + \gamma_j\, \ell n\, C_k + \ell n\, L_i + \ell n\, \varepsilon_{ijk}$$

which is a linear regression model with dependent variable $\ell n\, X_{ijk}$ and independent variable $\ell n\, C_k$. Details and justification for this model are discussed in the section "Comparison of Accuracy and Precision Across Water Types."

The natural logarithms of the individual laboratory's data were regressed against the natural logarithms of the true concentration levels for the six ampules in each water type. The predicted values $\ell n\, \hat{X}_{ijk}$ were obtained from the regression equation, and the missing values for X_{ijk} were estimated by $X_{ijk} = \exp(\ell n\, \hat{X}_{ijk})$, where exp(x) denotes the constant e raised to the power X.

If the ranking test rejected a laboratory for a specific water type, then all the laboratory's data for that water type were rejected as outliers. The rejected values were excluded from all the remaining analyses. In addition, after completion of the laboratory ranking procedure, the predicted values created to fill in for the missing data were rejected and excluded from further analyses for all laboratories.

Individual Outliers

The data remaining after the laboratory ranking procedure were grouped by water type. For each water type, the data were broken down into six subsets defined by the six concentration levels (ampules) used in the study. For each subset of the data, all missing, zero, "less than," and "nondetect" data were rejected. Next, the test for individual outliers constructed by Thompson(6), and suggested in the ASTM Standard Practice D2777-77, was applied to the data using a 5-percent significance level. If an individual data point was rejected based on this test, it was removed from the subset, and the test was repeated using the remaining data in the subset. This process was continued until no additional data could be rejected.

STATISTICAL SUMMARIES DATA

Several summary statistics were calculated using the data remaining for each concentration level after the outlier rejection tests were performed. These summary statistics include: the number of retained data points, the mean recovery, accuracy as a percent relative error, the absolute overall standard deviation, the percent relative overall standard deviation, the absolute single-analyst standard deviation, and the percent relative single-analyst standard deviation. The basic formulas used to calculate these statistics are presented below (X_1, X_2,..., X_n denote the values of the n retained data points for a specific concentration level):

Mean Recovery (X):

$$X = \frac{1}{n} \sum_{i=1}^{n} X_i$$

The usual concentration notation for mean recovery is $\bar{X}$. However, the symbol X is used in this report to be consistent with the IMVS output.

Accuracy as a Percent Relative Error:

$$\text{Percent RE} = \frac{X - \text{True Value}}{\text{True Value}} \times 100$$

Overall Standard Deviation:

$$S = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n} (X_i - X)^2}$$

and

Percent Relative Overall Standard Deviation:

$$\text{Percent RSD} = \frac{S}{X} \times 100$$

The overall standard deviation S indicates the precision associated with measurements generated by a group of laboratories. This represents the broad variation in the data collected in an interlaboratory study. However, a measure of how well an individual analyst can expect to perform in his own laboratory is another important measure of precision. This single-analyst precision, denoted by SR, was estimated for each Youden pair by

$$SR = \sqrt{\frac{1}{2(m-1)}\sum_{i=1}^{m} (D_i - \bar{D})^2}$$

where

m = the number of complete sets of Youden pair observations remaining after outliers have been removed,

D_i = the difference between the observations in the ith Youden pair

and

$\bar{D}$ = average of the D_i values.

The percent relative single-analyst standard deviation was calculated by

$$\text{Percent RSD-SR} = \frac{SR}{X^*} \times 100$$

where X* is the average of the two mean recovery statistics corresponding to the two concentration levels defining the particular Youden pair.

These summary statistics provide detailed information on the accuracy and precision of the data obtained for each concentration level. One objective of the statistical analysis of the data is to summarize the information about accuracy and precision which is contained in the statistics.

A systematic relationship often exists between the mean recovery (X) and the true concentration level (C) of the analyte in the sample. In addition there are often systematic relationships between the precision statistics (S and SR) and the mean recovery (X). Usually these systematic relationships can be adequately approximated by a linear relationship (i.e. by a straight line). Once these straight lines are established, they can be used to conveniently summarize the behavior of the method within a water type, and they can aid in comparing the behavior of the method across water types. In addition they can be used to obtain estimates of the accuracy and precision at any concentration level within the applicable range studied. They can also be used to predict the behavior of the method when used under similar conditions. These important relationships are discussed below.

STATEMENT OF METHOD ACCURACY

The accuracy of the method is characterized by the relationship of the mean recovery (X) to the true concentration (C) of the analyte in the water sample. In order to obtain a mathematical expression for this relationship, a regression line of the form

$$X = a + b \cdot C \qquad (1)$$

was fitted to the data by regression techniques.

The true concentration values often vary over a wide range. In such cases, the mean recovery statistics associated with the larger concentration values tend to dominate the fitted regression line producing relatively

larger errors in the estimates of mean recovery at the lower concentration values. In order to eliminate this problem, a weighted least squares technique was used to fit the mean recovery data to the true concentration values. The weighted least-squares technique was performed by dividing both sides of Equation (1) by C resulting in Equation (2)

$$\frac{X}{C} = a \cdot \frac{1}{C} + b \qquad (2)$$

The (X/C) values were regressed against the (1/C) values using ordinary least squares to obtain estimates for the values of a and b. (This is equivalent to performing a weighted least squares with weights $w = 1/C^2$; see Reference (7), page 108 for details.) Equation (2) can easily be converted to the desired relationship given by Equation (1). The intercept (b) from Equation (2) becomes the slope (b) for Equation (1) and slope (a) from Equation (2) becomes the intercept (a) for Equation (1). Equation (1) can be used to calculate the percent recovery over the applicable range of concentrations used in the study.

The percent recovery is given by

$$\text{Percent Recovery} = \left[\frac{a + b \cdot C}{C}\right] \times 100 = \left[\frac{a}{C} + b\right] \times 100$$

If the absolute value of the ratio (a/C) is small relative to the slope (b) for the low concentration levels used in the study, then the percent recovery can be approximated by b x 100. For example, suppose the true concentration values range from 25 µg/L to 515 µg/L, and the fitted line is given by $X = 0.20 + 0.85 \cdot C$. The percent recovery would be approximated by 0.85 x 100 = 85 percent over the specified range of 25 µg/L to 515 µg/L.

If the ratio (a/C) is not small relative to the slope (b), then the percent recovery depends upon the true concentration (C), and it must be evaluated at each concentration value within the specified range.

STATEMENT OF METHOD PRECISION

The precision of the method is characterized by the relationships between precision statistics (S and SR) and mean recovery (X). In order to obtain a mathematical expression for these relationships, regression lines of the form

$$S = d + e \cdot X \tag{4}$$

and

$$SR = f + g \cdot X^* \tag{5}$$

were fitted to the data by regression techniques.

As discussed previously with respect to accuracy, the values of X and X* often vary over a wide range. In such cases, the standard deviation statistics associated with the larger mean recovery values will dominate the regression lines. This will produce relatively larger errors in the estimates of S and SR at the lower mean recovery values. Therefore, a weighted least squares technique was also used to establish the values of the parameters d, e, f, and g in Equations (4) and (5). The weighted least squares technique was performed by dividing both sides of Equation (4) by X resulting in Equation (6)

$$\frac{S}{X} = d \cdot \frac{1}{X} + e \tag{6}$$

and by dividing both sides of Equation (5) by X* resulting in Equation (7)

$$\frac{SR}{X^*} = f \cdot \frac{1}{X^*} + g \tag{7}$$

The (S/X) values were regressed against the (1/X) values and the (SR/X*) values were regressed against the (1/X*) values using ordinary least squares to obtain estimates for the parameters d, e, f, and g.

Equations (4) and (5) were obtained from Equations (6) and (7) in a manner similar to that discussed for mean recovery. The slope (d) for Equation (6) is the intercept (d) for Equation (4), and the intercept (e) for Equation (6) is the slope (e) for Equation (4). Similarly, the slope (f) for Equation (7) is the intercept (f) for Equation (5), and the intercept (g) for Equation (7) is the slope (g) for Equation (5).

Given Equations (4) and (5), the percent relative overall standard deviation and the percent relative single-analyst standard deviation are

$$\text{Percent RSD} = \left[\frac{d}{\bar{X}} + e\right] \times 100 \tag{8}$$

and

$$\text{Percent RSD-SA} = \left[\frac{f}{\bar{X}^*} + g\right] \times 100 \tag{9}$$

respectively. If the absolute value of the ratio (d/X) is small relative to the slope (e), then the percent relative overall standard deviation can be approximated by (e x 100) over the applicable range of mean recovery values. Similarly if the ratio (f/X*) is small relative to the slope (g), then the percent relative single-analyst standard deviation can be approximated by (g x 100) over the applicable range of mean recovery values. If the ratios (d/X) and (f/X*) are not small relative to the slopes (e) and (f), then the percent relative standard deviations depend upon the values of the mean recovery statistics X and X*, and they should be evaluated separately for each value of X and X*.

COMPARISON OF ACCURACY AND PRECISION ACROSS WATER TYPES

It is possible that the accuracy and precision of Method 605 depend upon the type of water being analyzed. The summary statistics X, S, and SR are calculated separately for each concentration level within each water type. They can be compared across water types in order to obtain information about the effects of water type on accuracy and precision. However, the use of these summary statistics in this manner has several disadvantages. First, it is cumbersome since there are 36 mean recovery statistics (X) (6 concentrations x 6 waters), 36 precision statistics (S), and 18 precision statistics (SR) calculated for each compound. Comparison of these statistics across concentration levels and across water types becomes unwieldy. Second, the statistical properties of this type of comparison procedure are difficult to determine. Finally, due to variation associated with X, S, and SR, comparisons based on these statistics can lead to inconsistent conclusions about the effect of water type. For example,

distilled water may produce a significantly lower value than surface water for the precision statistic S at a high concentration, but a significantly higher value for S at a low concentration.

An alternative approach, described in detail(8) has been developed to test for the effects of water type. This alternative approach is based on the concept of summarizing the average effect of water type across concentration levels rather than studying the local effects at each concentration level. If significant differences are established by this alternative technique, then the summary statistics can be used for further local analysis.

The test for the effect of water type is based on the following statistical model. If X_{ijk} denotes the measurement reported by laboratory "i", for water type "j", and ampule k, then

$$X_{ijk} = \beta_j \cdot C_k^{\gamma_j} \cdot L_i \cdot \varepsilon_{ijk} \quad . \quad \begin{matrix} i = 1,2,\ldots, n \\ j = 1,2,\ldots, 6 \\ k = 1,2,\ldots, 6 \end{matrix} \quad . \qquad (10)$$

The model components β_j and γ_j are fixed parameters which determine the effect of water type "j" on the behavior of the observed measurements (X_{ijk}). The parameter C_k is the true concentration level associated with ampule k. If the observed measurements are plotted versus concentration on a log-log scale, the data will plot approximately along a straight line. The intercept for the line is log β_j and the parameter γ_j is the slope of the line. The model component L_i is a random factor which accounts for the systematic error associated with laboratory "i". The model component ε_{ijk} is the random factor which accounts for the intralaboratory error.

The model is designed to approximate the global behavior of the data. The multiplicative structure was chosen because of two important properties. First, since the model describes a straight line relationship on a log-log scale, it allows for a possible curvilinear relationship between the data (X_{ijk}) and the true concentration level C_k through the use of the exponent γ_j on C_k. This makes the model more flexible in comparison to straight line models. Second, as will be seen below, there is an inherent increasing relationship between the variability in the data and the concentration level C_k in this model. This property is important because it is typical of

interlaboratory data collected under conditions where the true concentration levels vary widely.

Accuracy is related directly to the mean recovery or expected value of the measurements (X_{ijk}). The expected value for the data modeled by Equation (10) is

$$E(X_{ijk}) = \beta_j \cdot C_k^{\gamma_j} \cdot E(L_i \cdot \varepsilon_{ijk}) \qquad (11)$$

Precision is related to the variability in the measurements (X_{ijk}). The variance of the data modeled by Equation (10) is

$$Var(X_{ijk}) = \left[\beta_j C_k^{\gamma_j}\right]^2 Var(L_i \cdot \varepsilon_{ijk}) \qquad (12)$$

which is an increasing function of C_k.

The effect of water type on the accuracy and precision of Method 605 is determined by the values of the parameters (β_j) and (γ_j) in Equations (11) and (12). If the (β_j) and (γ_j) vary with j (i.e. vary across water type), then the accuracy and precision of the method also vary across water type.

In order to determine if these parameters do vary across water type and to compare their values, they must be estimated from the laboratory data using regression techniques. Equation (10) represents the basic model. However, taking natural logarithms of both sides of Equation (10), the following straight line regression model is obtained

$$\ell n\, X_{ijk} = \ell n\, \beta_j + \gamma_j\, \ell n\, C_k + \ell n\, L_i + \ell n\, \varepsilon_{ijk} \qquad (13)$$

which can be analyzed using standard linear model analysis techniques. The parameter Ln β_j is the intercept and γ_j the slope of the regression line associated with water type "j". It is assumed that $\ell n\, L_i$ is normally distributed with mean 0 and variance σ_L^2, and that $\ell n\, \varepsilon_{ijk}$ is normally distributed with mean 0 and variance σ_ε^2, and that the ($\ell n\, L_i$) and ($\ell n\, \varepsilon_{ijk}$) terms are independent.

Based on Equation (13), the comparison of water types reduces to the comparison of straight lines. Distilled water is viewed as a control, and each of the remaining lines is compared directly to the line for distilled water.

Using the data on the log-log scale and regression techniques, the parameters $\ell n\ \beta_j$ (and hence β_j) and γ_j can be estimated. The estimates are then used to test the null hypothesis that there is no effect due to water type. The formal null and alternative statistical hypotheses H_0 and H_A are given by

$$H_0: \ \ell n\ \beta_j - \ell n\ \beta_1 = 0 \text{ and } \gamma_j - \gamma_1 = 0 \text{ for } j = 2,3,4,5,6$$

versus

$$H_A: \ \ell n\ \beta_j - \ell n\ \beta_1 \neq 0 \text{ and/or } \gamma_j - \gamma_1 = 0 \text{ for some } j = 2,3,4,5,6 \ .$$

The test of null hypothesis H_0 against the alternative hypothesis H_A is based on an F-statistic derived from standard linear model theory. The probability of obtaining a value of an F-statistic as large as the value which was actually observed (F OBS), denoted by P(F > F OBS), is calculated under the assumption that H_0 is true. The null hypothesis H_0 is rejected in favor of H_A if P(F > F OBS) is less than 0.05.

If H_0 is rejected, then some linear combination of the differences $\ell n\ \beta_j - \ell n\ \beta_1$ and $\gamma_j - \gamma_1$ is statistically different from zero. However, this does not guarantee there will be a statisticlaly significant direct effect attributable to any specific water type since the overall F test can be overly sensitive to a minor systematic effect common to several water types. The effect due to a specific water type is judged to be statistically significant only if one of the differences $\ell n\ \beta_j - \ell n\ \beta_1$ and/or $\gamma_j - \gamma_1$ is statistically different from zero. This is determined by checking the simultaneous 95-percent confidence intervals which are constructed for each of these differences. Each true difference can be stated to lie within its respective confidence interval with 95-percent confidence. If zero is contained within the confidence interval, then there is no evidence that the corresponding difference is significantly different from zero.

If at least one of the confidence intervals for the differences $\ell n\ \beta_j - \ell n\ \beta_1$ or $\gamma_j - \gamma_1$ fails to include zero, then the statistical significance of the effect due to water type has been established. However, establishment of a statistically significant effect due to water type does

not necessarily mean that the effect is of practical importance. Practical importance is related to the size and interpretation of the difference.

The interpretation of the differences involves comparing the mean recovery and standard deviation of the (X_{ijk}) data for each water type to the mean recovery and standard deviation obtained for distilled water. These comparisons are made on a relative basis. The mean recovery for water type "j" is given by Equation (11). The mean recovery for water type "j" is compared to that for distilled water (j=1) on a relative basis by the ratio

$$\frac{E(X_{ijk})}{E(X_{ilk})} = \frac{\beta_j C_k^{\gamma_j} E(L_i \cdot \varepsilon_{ijk})}{\beta_1 C_k^{\gamma_1} E(L_i \cdot \varepsilon_{ilk})} = \frac{\beta_j}{\beta_1} C_k^{\gamma_j - \gamma_1} \qquad (14)$$

(The ratio of the standard deviations would be equivalent to Equation (14) and therefore the interpretation of the effect on precision is the same as that for the effect on mean recovery.)

The ratio in Equation (14) is a measure of the relative difference in mean recovery between water type "j" and distilled water. It is composed of two parts (a) β_j/β_1, which is independent of the true concentration level (i.e. the constant bias), and (b) $C_k^{\gamma_j - \gamma_1}$ which depends upon the true concentration level (i.e. the concentration dependent bias). If $\gamma_j - \gamma_1$ is zero, then the relative difference in mean recovery is just β_j/β_1, which is independent of concentration level C_k. It can then be stated that the mean recovery of water type "j" is (β_j/β_1) x 100 percent of the mean recovery for distilled water. If $\gamma_j - \gamma_1$ is not zero, then the mean recovery of water type "j" is $((\beta_j/\beta_1).C_k^{\gamma_j - \gamma_1})$ x 100 percent of that for distilled water, and therefore depends upon the true concentration level C_k.

In order to illustrate these points, consider the following example. Suppose that a significant F-value has been obtained, and the confidence intervals for all the differences contain zero except for water type 5. Suppose for water type 5, the point estimate for $\ln \beta_5 - \ln \beta_1$ is -0.38 and the confidence interval for $\ln \beta_5 - \ln \beta_1$ is (-0.69, -0.07). Suppose the point estimate for $\gamma_5 - \gamma_1$ is 0.07, and the confidence interval for $\gamma_5 - \gamma_1$ is (-0.04, 0.18). In this case, a statistically significant effect due to water type has been established which involves only water type 5. The practical significance of this effect is judged by considering Equation

(14). The ratio of mean recoveries for water type 5 and distilled water is given by

$$\frac{E(X_{i5k})}{E(X_{i1k})} = \frac{\beta_5}{\beta_1} C_k^{\gamma_5 - \gamma_1} \tag{15}$$

and the ratio of the standard deviations is given by

$$\sqrt{\frac{Var(X_{i5k})}{Var(X_{i1k})}} = \frac{\beta_5}{\beta_1} C_k^{\gamma_5 - \gamma_1} \tag{16}$$

Since the confidence interval for $\gamma_5 - \gamma_1$ contains zero, this difference is assumed to be insignificant and is set to zero. Therefore, Equations (15) and (16) reduce to β_5/β_1. The point estimate for $\ln \beta_5 - \ln \beta_1$ was -0.38. Therefore, the point estimate for β_5/β_1 is 0.68, and the mean recovery for water type 5 is estimated to be 68 percent of the mean recovery for distilled water. Similarly, the standard deviation for the data for water type 5 is estimated to be 68 percent of the standard deviation for distilled water. Since the 95 percent confidence interval for $\ln \beta_5 - \ln \beta_1$ was (-0.69,-0.07), any value in the interval (0.50, 0.93) is a reasonable estimate for β_5/β_1, and the mean recovery (standard deviation) for water type 5 can be claimed to be from 50 percent to 93 percent of the mean recovery (standard deviation) for distilled water. The practical significance of the effect due to water type 5 would depend upon the importance of a mean recovery (standard deviation) which is between 50 percent and 93 percent of the mean recovery (standard deviation) observed for distilled water.

The comparison of accuracy and precision across water types just discussed, is based on the assumption that Equation (10) approximately models the data. It is clear that in practical monitoring programs of this type such models cannot represent the data completely in every case. This analysis, therefore, is viewed as a screening procedure which identifies those cases where differences in water types are likely to be present. A more detailed, local analysis can then be pursued using the basic summary statistics for precision and accuracy.

SECTION 5

RESULTS AND DISCUSSION

The statistical analyses procedures described in Section 4 were applied to the raw data shown in Appendix C for the 17 laboratories participating in this interlaboratory study of Method 605. The results of those analyses are discussed in this section.

STATISTICAL SUMMARIES

The basic summary statistics discussed in Section 4 were calculated for the compounds, benzidine and DCB, and are summarized in Tables 4 and 5. The number of outliers for each data set as determined by the ranking and individual outlier test ranged from one to nine, and averaged about four outliers per data set. Twenty-four percent of the data for the two compounds were rejected.

REGRESSION EQUATIONS

Using the values contained in the summary statistics for the true concentrations, mean recoveries(X and X*), overall standard deviations (S), and single-analyst standard deviations (SR), the regression lines given by Equations 1, 4, and 5 were obtained for each compound and water type. These regression lines were summarized in Table 1 (see Section 2, page 6).

The slopes of the regression equations can be used to estimate the percentage recovery, and overall and single-analyst percent relative standard deviations at concentrations exceeding 20 µg/L since ignoring the intercepts introduces an error of less than 11 percent in the estimated value. Below 20 µg/L, some regression equations, for example, benzidine in tap water, have intercepts which are rather significant and care should be exercised in using the slopes as an estimate of accuracy and precision.

TABLE 4. STATISTICAL SUMMARY FOR BENZIDINE ANALYSES BY WATER TYPE

	WATER 1		WATER 2		WATER 3		WATER 4		WATER 5		WATER 6	
LOW YOUDEN PAIR	3	6	3	6	3	6	3	6	3	6	3	6
NUMBER OF DATA POINTS	13	12	11	12	10	11	12	12	8	8	10	8
TRUE CONC (C) UG/L	1.00	1.40	1.00	1.40	1.00	1.40	1.00	1.40	1.00	1.40	1.00	1.40
MEAN RECOVERY (X)	0.80	0.98	0.87	1.39	0.67	0.64	0.77	0.63	0.45	0.66	0.56	0.62
ACCURACY(%REL ERROR)	-19.74	-30.01	-13.20	-0.71	-32.61	-54.44	-22.75	-54.80	-54.54	-52.98	-43.50	-55.68
OVERALL STD DEV (S)	0.58	0.47	0.61	1.01	0.48	0.37	0.48	0.26	0.24	0.25	0.35	0.39
OVERALL REL STD DEV, %	71.84	48.26	70.32	72.79	71.11	57.44	62.28	40.75	51.99	38.03	62.76	62.18
SINGLE STD DEV, (SR)	0.44		0.78		0.29		0.32		0.09		0.24	
ANALYST REL DEV, %	49.63		69.10		43.67		45.56		16.61		40.30	
MEDIUM YOUDEN PAIR	1	5	1	5	1	5	1	5	1	5	1	5
NUMBER OF DATA POINTS	14	14	15	14	13	13	12	12	10	9	11	11
TRUE CONC (C) UG/L	12.00	8.00	12.00	8.00	12.00	8.00	12.00	8.00	12.00	8.00	12.00	8.00
MEAN RECOVERY (X)	8.71	5.88	8.07	4.79	7.28	4.54	5.89	4.75	7.20	5.27	5.55	3.74
ACCURACY(%REL ERROR)	-27.45	-26.50	-32.77	-40.18	-39.30	-43.21	-50.90	-40.64	-40.02	-34.14	-53.78	-53.25
OVERALL STD DEV (S)	3.41	1.39	4.01	2.39	3.91	3.30	4.38	3.05	6.20	2.48	4.12	2.69
OVERALL REL STD DEV, %	39.13	23.72	49.75	50.00	53.66	72.59	74.32	64.15	86.21	47.02	74.31	71.81
SINGLE STD DEV, (SR)	1.89		2.37		1.69		2.70		1.99		1.43	
ANALYST REL DEV, %	25.92		36.81		28.49		50.66		31.86		30.80	
HIGH YOUDEN PAIR	4	2	4	2	4	2	4	2	4	2	4	2
NUMBER OF DATA POINTS	15	15	15	15	13	14	13	14	9	10	11	12
TRUE CONC (C) UG/L	40.00	55.00	40.00	55.00	40.00	55.00	40.00	55.00	40.00	55.00	40.00	55.00
MEAN RECOVERY (X)	26.23	40.50	23.61	39.75	24.37	30.12	23.02	33.48	23.21	34.23	23.92	31.46
ACCURACY(%REL ERROR)	-34.42	-26.36	-40.98	-27.73	-39.09	-45.24	-42.45	-39.12	-41.96	-37.75	-40.21	-42.79
OVERALL STD DEV (S)	14.12	19.39	13.12	19.07	13.58	13.68	14.80	22.00	15.65	14.18	13.35	18.09
OVERALL REL STD DEV, %	53.84	47.89	55.60	47.97	55.73	45.42	64.29	65.71	67.41	41.41	55.80	57.49
SINGLE STD DEV, (SR)	11.08		10.97		8.81		9.03		9.60		5.75	
ANALYST REL DEV, %	33.22		34.63		32.36		31.97		33.41		20.76	

WATER LEGEND

1 - DISTILLED WATER
2 - TAP WATER
3 - SURFACE WATER
4 - WASTEWATER (C-74)
5 - WASTEWATER (C-75)
6 - WASTEWATER (C-76)

TABLE 5. STATISTICAL SUMMARY FOR DCB ANALYSES BY WATER TYPE

	WATER 1		WATER 2		WATER 3		WATER 4		WATER 5		WATER 6	
LOW YOUDEN PAIR	3	6	3	6	3	6	3	6	3	6	3	6
NUMBER OF DATA POINTS	15	12	13	15	14	16	9	11	12	12	11	9
TRUE CONC (C) UG/L	1.00	1.40	1.00	1.40	1.00	1.40	1.00	1.40	1.00	1.40	1.00	1.40
MEAN RECOVERY (X)	0.98	0.98	0.78	0.98	0.84	0.75	0.51	0.70	0.75	1.15	0.71	0.62
ACCURACY(%REL ERROR)	-2.45	-29.68	-22.31	-30.00	-16.09	-46.36	-48.67	-49.77	-24.59	-18.07	-28.84	-55.55
OVERALL STD DEV (S)	0.59	0.18	0.48	0.49	0.49	0.36	0.39	0.57	0.50	0.79	0.38	0.17
OVERALL REL STD DEV, %	60.47	18.31	61.45	49.97	58.85	47.45	75.15	81.42	65.99	68.66	52.80	75.82
SINGLE STD DEV, (SR)	0.32		0.32		0.33		0.44		0.50		0.31	
ANALYST REL DEV, %	32.67		36.06		41.03		72.50		53.12		46.56	
MEDIUM YOUDEN PAIR	1	5	1	5	1	5	1	5	1	5	1	5
NUMBER OF DATA POINTS	16	16	15	16	16	16	13	13	12	12	13	12
TRUE CONC (C) UG/L	15.00	11.00	15.00	11.00	15.00	11.00	15.00	11.00	15.00	11.00	15.00	11.00
MEAN RECOVERY (X)	10.49	6.81	9.83	6.35	8.96	6.27	5.99	5.18	8.10	8.09	8.53	5.21
ACCURACY(%REL ERROR)	-30.04	-38.11	-34.49	-42.28	-40.29	-43.00	-60.04	-52.88	-45.99	-26.42	-43.16	-52.67
OVERALL STD DEV (S)	4.92	2.80	3.90	2.59	4.04	2.93	4.05	2.97	5.75	5.46	4.33	3.01
OVERALL REL STD DEV, %	46.87	41.18	39.71	40.80	45.09	46.67	67.62	57.34	71.00	67.49	50.85	57.77
SINGLE STD DEV, (SR)	3.91		2.78		2.06		1.94		2.57		3.56	
ANALYST REL DEV, %	45.23		34.35		27.02		34.70		31.77		51.92	
HIGH YOUDEN PAIR	4	2	4	2	4	2	4	2	4	2	4	2
NUMBER OF DATA POINTS	16	16	16	16	15	16	13	14	12	13	13	14
TRUE CONC (C) UG/L	55.00	70.00	55.00	70.00	55.00	70.00	55.00	70.00	55.00	70.00	55.00	70.00
MEAN RECOVERY (X)	39.90	46.01	36.46	47.22	37.96	38.72	29.74	35.16	35.89	39.00	31.22	39.82
ACCURACY(%REL ERROR)	-27.46	-34.27	-33.70	-32.55	-30.97	-44.68	-45.93	-49.77	-34.75	-44.28	-43.23	-43.11
OVERALL STD DEV (S)	10.76	17.79	14.29	18.08	12.90	15.27	16.92	16.30	18.54	17.27	15.39	19.69
OVERALL REL STD DEV, %	26.98	38.67	39.19	38.29	33.98	39.43	56.89	46.36	51.67	44.28	49.29	49.45
SINGLE STD DEV, (SR)	13.75		10.67		10.25		7.98		8.88		10.57	
ANALYST REL DEV, %	32.01		25.50		26.72		24.58		23.71		29.75	

WATER LEGEND

1 - DISTILLED WATER
2 - TAP WATER
3 - SURFACE WATER
4 - WASTEWATER (C-74)
5 - WASTEWATER (C-75)
6 - WASTEWATER (C-76)

Based upon the slopes of the equations at concentrations above 20 µg/L, percentage recoveries for benzidine and DCB ranged from 52-70 and 48-66, respectively, for all water types. The overall percent relative standard deviation for benzidine and DCB ranged from 40-69 and 38-58 percent, respectively, for all water types. The single-analyst percent relative standard deviation for benzidine and DCB ranged from 24-40 and 26-40, respectively, for all water types.

EFFECTS OF WATER TYPE

The multiplicative model (Equation 10 in Section 4) was fitted separately to the data for each compound in order to test for the effects due to water type. These analyses are summarized in Table 6. The detailed analyses of variance tables supporting these results are presented in Appendix D.

TABLE 6. SUMMARY OF THE TESTS FOR THE DIFFERENCES ACROSS WATER TYPES

Compound	Observed F-Value	P[F > F OBS]	F-Test Statistically Significant at the 5% Level?	Significance Effect Established by the 95% Confidence Intervals?	Practical Significance Established
Benzidine	1.78	0.06	NO		
DCB	4.88	0.00	YES	YES	NO

There was no evidence of a statistically significant effect on accuracy or precision due to water type for benzidine. However, in the case of DCB, a statistically significant effect was established since $P[F > F\ OBS] \leq 0.05$. The simultaneous confidence intervals for $\ell n\ \beta_j - \ell n\ \beta_1$ and $\gamma_j - \gamma_1$ all included zero except for the fourth wastewater, C-74. In that case the confidence interval for $\gamma_4 - \gamma_1$ included zero, but the confidence interval for $\ell n \beta_4 - \ell n\ \beta_1$ failed to cover zero. This indicated that the statistically significant effect was associated with the C-74 wastewater.

Review of the summary statistics for DCB in Table 5 indicates that the effect is due to the low recovery of DCB from wastewater C-74 relative to

distilled water. However, the observed effect is not considered to be of practical importance for the following reasons:

(1) The broad range (24-73%) of single-analyst precision values for DCB in all water types minimizes the significance of the lower DCB recovery observed for C-74.

(2) The overall precision and single-analyst precision statistics S and SR for wastewater C-74 did not display any consistent pattern relative to the precision statistics for distilled water. As shown in Table 5, the overall precision, S, is smaller for wastewater C-74 relative to distilled water for ampules 1, 3, 5 and 6, but larger for ampules 2 and 4. Further, the single-analyst precision, SR, is smaller for wastewater C-74 for the medium and high Youden pair, but larger for the low Youden pair relative to distilled water.

The reasons for the lower recovery of DCB in wastewater C-74 are not known. However, one could speculate that since the effluent had been treated by carbon filtration (and the other samples had not) some DCB loss due to irreversible adsorption onto fine carbon particles might occur. Clearly there is not enough evidence to support this conclusion, although DCB is highly adsorptive from aqueous solution, as compared to benzidine. This property of DCB should be considered when applying the method to various water types.

SINGLE LABORATORY DATA - BATTELLE

Before discussing the data obtained in this study from an analytical chemist's viewpoint, it seems useful to review the single laboratory performance characteristics of the method when performed at Battelle. Under separate contract with USEPA, Battelle evaluated the method's detection limit in distilled water and in two of the three wastewaters employed in the interlaboratory study. In the same study, Battelle examined the accuracy and precision of the method at various concentration levels. A summary of the results of that study (9) is given below:

- Method detection limits are 0.1 µg/L for both benzidine and DCB in reagent water, pigment manufacturing effluent, and aniline manufacturing effluent. For both compounds the relative standard deviation were approximately 10 percent and recoveries were greater than 55 percent at the 0.5 µg/L level in reagent water, as well as in two of the manufacturing wastewaters used in this study.

- Recoveries for benzidine are better than 85 percent at levels greater than 2 µg/L and decrease to 60-70 percent at the 0.5 µg/L level. Recoveries for DCB are 70-75 percent at levels greater than 2 µg/L and decrease to 55 percent at the 0.5 µg/L level.

- The maximum recovery of 75 percent for DCB is a result of its limited extraction efficiency into 1 M sulfuric acid from chloroform. Losses for benzidine and DCB at the lower concentrations are probably due to oxidation.

- Determination of benzidine at 0.6 Volts vs. Ag/AgCl is very useful in reducing interference problems when analyzing actual wastewaters. Even lower potentials may be useful with certain electrochemical detectors. The lowest potential demonstrating a good response for benzidine should be employed for complex wastewaters.

A summary of the method detection limit data for the benzidines is given in Table 7. The data obtained in the MDL study agree very well with the values estimated and reported in the original Method 605 draft. Figure 1 demonstrates the performance of the method for the pigment ink manufacturing effluent. Operation of the detector at low potential (0.6 volts) for benzidine detection eliminated interference problems (as illustrated in Figure 1).

TABLE 7. METHOD DETECTION LIMITS FOR METHOD 605

Compound	Originally Reported in Method 605 Draft	MDL (µg/L) Reagent Water	Wastewater C-74(a)	Wastewater C-75(b)
Benzidine	0.05	0.08	0.19	0.06
DCB	0.1	0.13	0.22	0.09

(a) A final effluent from an aniline production facility.

(b) A final effluent from a pigment manufacturing facility.

GENERAL DISCUSSION OF METHOD PERFORMANCE

The percent recovery and precision data for benzidine and DCB are given in the statistical summaries (Tables 4 and 5 respectively). Average percent recoveries for benzidine ranged between 44-99 percent and were independent of concentration. Recoveries for distilled water were 65-80 percent, whereas recoveries from industrial effluents were somewhat lower (44-77 percent). Recoveries for DCB ranged between 40-98 percent and were independent of concentration. Recoveries for distilled water and industrial effluent samples were similar, except that C-74 gave a somewhat lower recovery for DCB. Recoveries for both benzidine and DCB from finished drinking water and distilled water were similar, which indicates that the dechlorination procedure was effective.

Overall precision ranged between 24-86 percent for benzidine and 18-81 percent for DCB for the various water types, and was somewhat larger for the low Youden pair than for the medium and high Youden pairs. Single-analyst precision for the various water types ranged between 17-69 percent for benzidine and 24-72 percent for DCB. However, the single-analyst precision at the highest Youden pair (approximately 50 µg/L) ranged between 21-35 percent for both benzidine and DCB, and was essentially independent of water type.

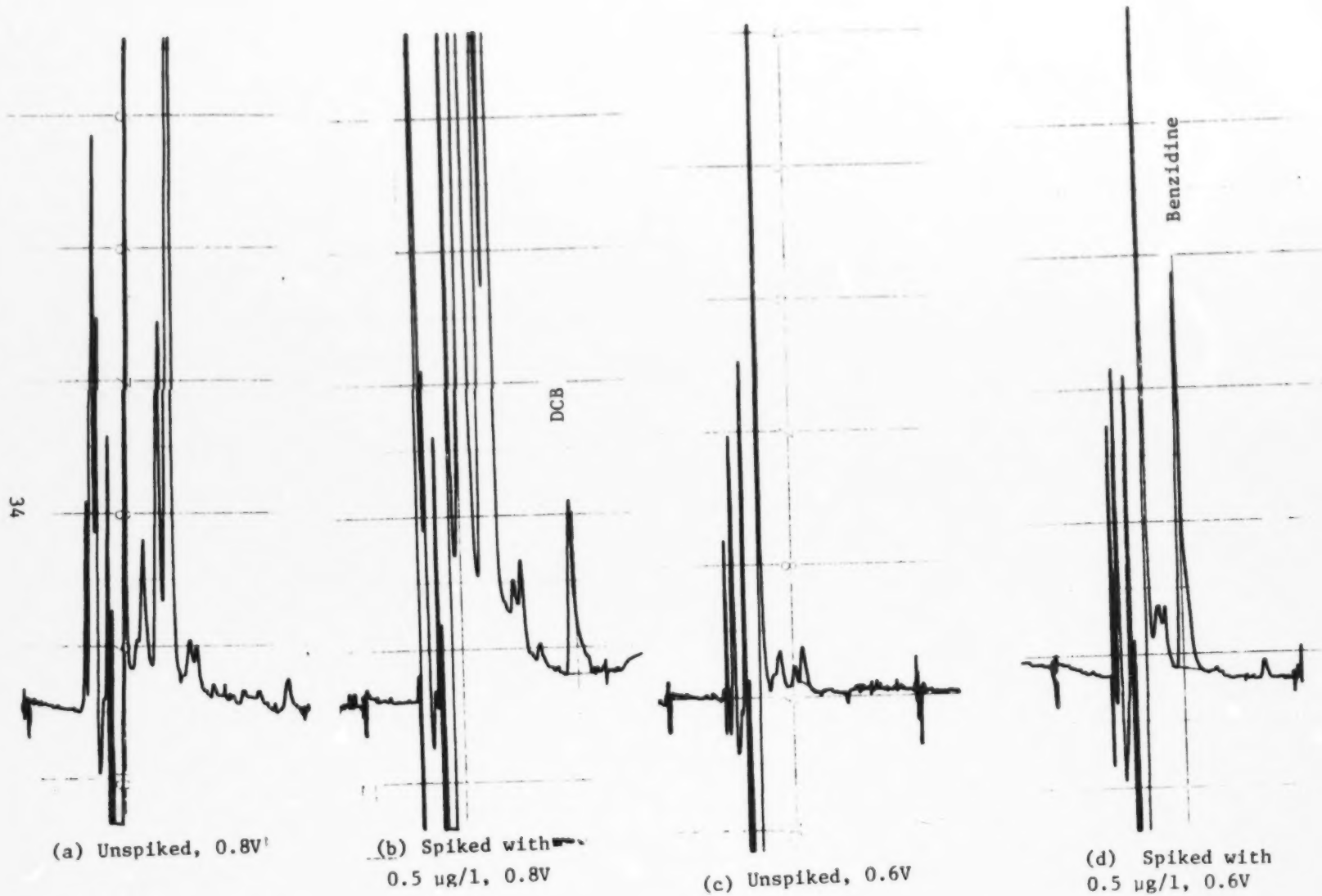

Figure 1. MDL Determination for Pigment Ink Manufacturing Wastewater

Examination of the raw data from each of the laboratories (Appendix C) reveals that several of the laboratories (#101, #103, #109, and #116) did not obtain good recovery for benzidine in any of the matrices, even at the highest level, while many of the other laboratories obtained consistently good recovery for benzidine. A similar pattern for DCB was not found. This observation seems to indicate that a source of systematic error at several of the laboratories is contributing to the poor interlaboratory precision especially for benzidine.

There are several possible sources of error that may account for the poor precision data. DCB and, particularly, benzidine are readily oxidized and could be readily destroyed during sample workup by oxidants present in either the sample itself or in some of the reagents. One laboratory (#101) used a dichromate glassware washing procedure, which may have resulted in their poor recovery for benzidine. Although residual dichromate may not have been the only factor in this instance, it is recommended that dichromate rinsing of glassware be avoided because of the strong oxidizing capability of dichromate.

Residual oxidant (e.g. chlorine) in the reagent water supply is another potential source of imprecision in the method. At Battelle, the reagent water used for Method 605 has been from a Millipore "Milli-Q" system, which utilized reverse osmosis, ion-exchange, and carbon filtration purification steps. Carbon filtration is particularly important because it removes residual chlorine. Most of the laboratories in the study used a water purification system similar to the Millipore system. However, several of the laboratories (#106, #109, #113, and #116) used bottled water, purchased commercially, without stating its quality. One laboratory (#101) used "HPLC" grade water, which is believed to be at least equal in quality to the "Milli-Q" type system. One laboratory (#103) did not specify the type of water used. Oxidants present in some of the reagent water sources may have contributed to the low benzidine recoveries and large imprecision obtained at the laboratories. Reagent water used for blanks as well as for preparing reagents for sample workup and HPLC mobile phase must be free from even traces of oxidant, as demonstrated by obtaining good recovery of benzidine

from spiked process blanks. Oxidant contamination of solvents and other reagents is also a possible source of error that needs to be considered.

Another source of imprecision in the method is the performance of the HPLC separation and detection system. The method employs a buffered, reverse-phase separation system and an electrochemical detection system. Many of the laboratories were inexperienced in utilizing buffered, reversed -phase systems and only a few had previous experience with the electrochemical detection system. Several laboratories experienced problems with deterioration of the Teflon sleeve on the reference electrode for the electrochemical detector. This problem was solved either by obtaining another electrode from the manufacturer or by placing an additional heat shrink Teflon sleeve over the existing one. The sensitivity of the electrochemical detector in all of the laboratories appeared to be adequate for detecting the lowest Youden pair. Variation in detector response was not found to be a major problem, although daily or more frequent calibration was required and a few laboratories found it necessary to polish the glassy carbon working electrode every few days.

HPLC separation efficiency was generally not as good as has been routinely obtained at Battelle ($\sim$5,000 theoretical plates per 25-cm column) and in some cases, was very poor ($<$1000 theoretical plates per 25 cm column). Resolution of analytes from interferences becomes a significant problem when such low separation efficiencies are achieved. Probably the method should specify that a minimum separation efficiency (e.g. $>$3000 theoretical plates per 25-cm column) be achieved before sample analyses are conducted.

Several of the laboratories objected to the large number of manipulations required in the method. This is a valid consideration, although elimination of any of the present steps in the procedure does not appear feasible.

The exact sources of imprecision in the data are not known, although a number of possibilities have been identified. Probably the imprecision is due to the cumulative effect of at least the following facets of the procedure:

- Benzidine and DCB are readily oxidized and sources of oxidant must be carefully avoided.
- The basic character of the compounds requires careful pH control throughout the workup procedure.
- The operational characteristics of the electrochemical detector, while relatively straighforward, are not recognized as widely as the UV or other common HPLC detectors.

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APPENDIX A

BENZIDINES

METHOD 605

1. Scope and Application

1.1 This method covers the determination of selected benzidine derivatives. The following parameters may be determined by this method:

Parameter	STORET No.
Benzidine	39120
DCB	34631

1.2 This method is applicable to the measurement of these compounds in municipal and industrial discharges. It is designed to be used to meet the monitoring requirements of the National Pollutant Discharge Elimination System (NPDES). As such, it presupposes a high expectation of finding the specific compounds of interest. If the user is attempting to screen samples for any or all of the compounds above, he must develop independent protocols for the verification of identifications implied with the use of these techniques.

1.3 The sensitivity of this method is usually dependent upon the level of interferences rather than instrumental limitations. The limits of detection listed in Table A-1 represent sensitivities that can be achieved in wastewaters under ideal conditions.

1.4 This method is recommended for use only by experienced residue analysts or under the close supervision of such qualified persons.

2. Summary of Method

2.1 Benzidine and DCB are extracted from the sample at pH 7-8 using chloroform. The extract is then back extracted into acid, reextracted into chloroform at neutral pH and concentrated. The

TABLE A-1. LIQUID CHROMATOGRAPHY OF BENZIDINES ON LICHROSORB RP-2

Compound	Retention Time, Minutes	Detection Limit, (µg/L)
Benzidine	6.1	0.05
3,3'-dichlorobenzidine	12.1	0.1

Column conditions: Lichrosorb RP-2, 5 micron particle diameter, packed in a 25 cm x 4.6 mm ID stainless steel column. Flow rate: 0.8 mL/min. Mobile phase: 50% acetonitrile - 50% 0.1 M pH 4.7 acetate buffer.

Detection limit is calculated from the minimum detectable response of the electrochemical detector being equal to five times the background noise, assuming a 5 mL final extract volume of the 500 mL sample, and assuming in injection volume of 25 microliters.

benzidines are determined in the final extract using high performance liquid chromatography (HPLC) with electrochemical detection.

2.2 If interferences are encountered with the measurement of benzidine, the method provides additional detector settings to increase the selectivity of the analytical system.

3. Interferences

3.1 Although the detection system is highly selective, solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

3.2 Interferences coextracted from the samples will vary considerably from source to source, depending upon the diversity of the industrial complex or municipality being sampled. While general cleanup techniques are provided as part of this method, unique samples may require additional cleanup approaches to achieve the sensitivities stated in Table A-1.

3.3 Some dye plant effluents contain large amounts of components with retention times in the vicinity of benzidine. In these cases, it has been found useful to reduce the electrode potential in order to eliminate the interferences but still detect the benzidine.

4. Apparatus and Materials

4.1 Sampling equipment, for discrete or composite sampling.

4.1.1 Grab sample bottle - amber glass, pint or quart volume. Quart bottles should be only half filled in the field. French or Boston Round design is recommended. The container should be washed and solvent rinsed before use to minimize interferences.

4.1.2 Bottle caps - Threaded to screw on sample bottles. Caps must be lined with Teflon. Foil may be substituted if sample is not corrosive.

4.1.3 Compositing equipment - Automatic or manual compositing system. Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated during sampling. No tygon or rubber tubing may be used in the system.

4.2 Separatory funnel 1000-mL and 250-mL, with Teflon stopcock.

4.3 Drying column - A 20 mm ID pyrex chromatographic column with coarse frit.

4.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

4.5 Rotating evaporator.

4.6 100-mL round bottom flasks with 24/40 joints.

4.7 15-mL conical screw capped graduated centrifuge tubes with Teflon lined caps.

4.8 Pasteur pipettes and bulbs.

4.9 High performance liquid chromatography - Analytical system complete with column supplies, recorder, syringes, and the following components:

4.9.1 Solvent delivery system, with pulse damper (Altex 110A or equivalent).

4.9.2 Injection valve (Water's U6K or equivalent).

4.9.3 Electrochemical detector (Bioanalytical Systems LC-2A with glassy carbon electrode or equivalent)

5. Reagents

5.1 Preservatives:

5.1.1 Sodium hydroxide - (ACS) 10 N in distilled water.

5.1.2 Sulfuric acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

5.1.3 Sodium thiosulfate - (ACS) granular.

5.2 Sulfuric Acid (1M) - (ACS) 58 mL/liter in distilled water.

5.3 Sodium Hydroxide (20%) - ACS) 20 grams/100 mL in distilled water.

5.4 Sodium Hydroxide (1M) - (ACS) 40 grams/liter in distilled water.

5.5 Sodium tribasic phosphate (0.4M) - (ACS) 160 grams Na_3PO_4 $12H_20$ in 1 liter of distilled water.

5.6 Acetate buffer 0.1M, pH 4.7; 5.8 mls glacial acetic acid (ACS) and 13.6 grams of sodium acetate trihydrate (ACS) per liter in distilled water.

5.7 Acetonitrile - Pesticide quality or equivalent.

5.8 Methyl alcohol - Pesticide quality or equivalent.

5.9 Chloroform (preserved with 1% ETOH) - Burdick and Jackson

5.10 Water - Purified (e.g. from Millipore RO-4 System or equivalent).

5.11 HPLC mobile phase - Place 1 liter of filtered (through Millipore type FH filter, or equivalent) acetonitrile and 1 liter of filtered (through Millipore type GS filter, or equivalent) acetate buffer in a narrow mouth, glass, one-gallon jug and mix thoroughly. Prepare fresh weekly.

5.12 Stock standards - Prepare stock standard solutions at a concentration of 0.100 µg/µL by dissolving 0.0100 grams of assayed reference material in pesticide quality methyl alcohol and diluting to volume in a 100-mL ground glass stoppered volumetric flask. The stock solution is transfered to ground glass stoppered reagent bottles, stored in a refrigerator, and checked frequently for signs of degradation or evaporation, especially just prior to preparing working standards from them.

6. Calibration

6.1 Prepare calibration standards that contain the compounds of interest, either singly or mixed together. The standards should be prepared from the stock standards at the concentrations that will bracket the working range of the chromatographic system:

Solution Conc. (ng/µL)	Sensitivity (A Full Scale)
0.05	10
0.10	10
0.50	50
1.0	100
5.0	500

6.2 Assemble the necessary liquid chromatographic apparatus and establish operating parameters equivalent to those indicated in Table A-1.

6.3 A constant injection volume of 25 microliters should be employed for all subsequent measurements.

6.4 In order to determine the precision of the HPLC system, a series of 6 replicate injections of a 1 ng/μL solution of benzidine and DCB should be made on the 50nA full scalesetting. A precision of ±4% should be achieved for the peak heights of both benzidine and DCB. This measurement should be made every few weeks or whenever instrument related problems are apparent. A sample chromatogram is shown in Figure A-1.

6.5 Retention times should remain relatively constant (within ±5% day to day) with benzidine being 6.1 minutes and DCB being 12.1 minutes under the specified conditions. These values should be checked daily when the calibration injections are made.

6.6 If serious loss of response occurs, it may be necessary to polish the surface of the carbon electrode (according to the instructions supplied with the polishing kit). In this case, it will be necessary to recalibrate the system by injecting 0.1 and 1 ppm solutions.

6.7 When leaving the instrument idle, it is advisable to maintain a flow of 0.1 mL/min of mobile phase through the HPLC column in order to prolong column life.

7. Quality Control

7.1 Before processing any samples, the analyst should demonstrate through the analysis of a distilled water method blank, that all glassware and reagents are interference-free. Each time a set of samples is extracted or there is a change in reagents, a method blank should be processed as a safeguard against laboratory contamination.

7.2 Standard quality assurance practices should be used with this method. Field replicates should be collected to validate the precision of the sampling technique. Laboratory replicates should be analyzed to validate the precision of the analysis. Fortified samples should be analyzed to validate the accuracy of the analysis. Where doubt exists over the identification of a peak on

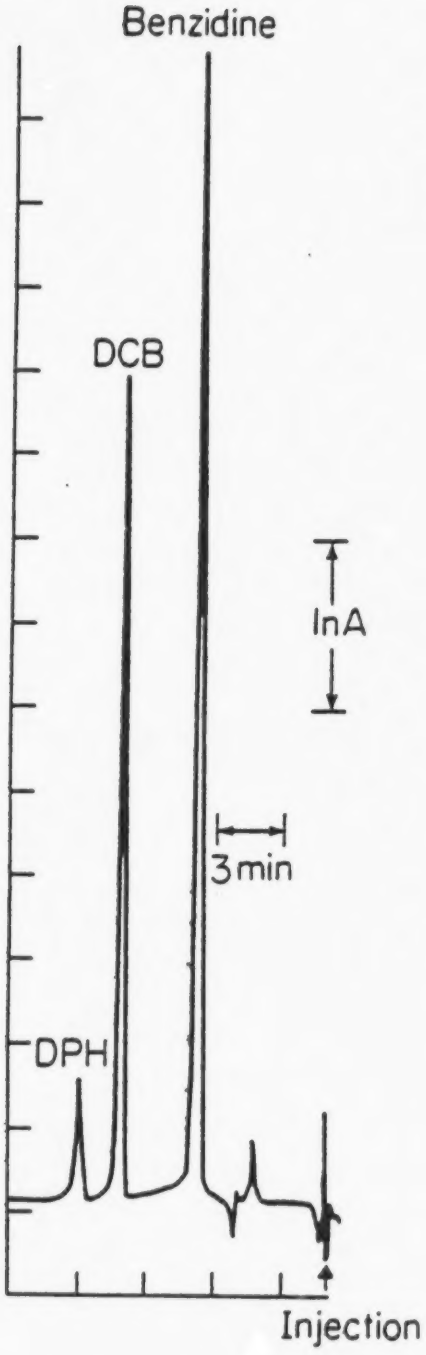

Figure A-1. Electrochemical Detection HPLC of Benzidines on Lichrosorb RP-2.For Conditions, See Table A-1.

the chromatogram, confirmatory techniques such as mass spectroscopy should be used.

8. Sample Collection, Preservation, and Handling

8.1 Grab samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be free of tygon and other potential sources of contamination.

8.2 The samples must be iced or refrigerated from the time of collection until extraction. Benzidine and dichlorobenzidine are easily oxidized by materials such as free chlorine. For chlorinated wastes, immediately add 35 mg sodium thiosulfate per part per million of free chlorine per liter.

8.3 If 1,2-diphenylhydrazine is likely to be present, adjust the pH of the sample to 4 $\pm$ 0.2 units to prevent rearrangement to benzidine. Otherwise, if the samples will not be extracted within 48 hours of collection, the sample should be adjusted to a pH range of 2.0 - 3.0 with sodium hydroxide or sulfuric acid.

8.4 All samples must be extracted within seven days and completely analyzed within 30 days of collection.

9. Sample Extraction

9.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Quantitatively pour the entire sample into a one-liter separatory funnel. Check the pH with wide-range paper and adjust to within the range of 6.5 - 7.5 with sodium hydroxide or sulfuric acid.

9.2 Add 100-mL chloroform to the sample bottle, and shake 30 seconds to rinse the walls. Transfer the solvent into the separatory funnel, and extract the sample by shaking the funnel for two minutes. Allow the organic layer to separate from the sample a minimum of 10 minutes, then collect the chloroform extract in a 250-mL separatory funnel.

9.3 Add a 50-mL volume of chloroform to the sample bottle and complete the extraction procedure a second time, combining the extracts in the separatory funnel. Perform a third extraction in the same manner with an additional 50-mL volume.

9.4 Discard any aqueous layer from the 250-mL funnel containing the combined organic layers. Add 25 mL of 1 M sulfuric acid and extract the sample by shaking the funnel for two minutes. Transfer the aqueous layer to a 250-mL beaker. Extract with two additional 25-mL portions of 1 M sulfuric acid and combine the acid extracts in the beaker.

9.5 Place a stirbar in the 250-ml beaker and stir the acid extract while carefully adding 5 mL of a .4M sodium tribasic phosphate. Then neutralize the extract to pH 6-7 by dropwise addition of 20 percent NaOH while stirring solution vigorously. Approximately 25-30 mL of 20 percent NaOH will be required and it should be added over at least a 2-minute period. Do not allow the sample pH ever to exceed pH 8.

9.6 Pour the neutralized extract into a 250-mL separatory funnel. Add 30 mL of chloroform and shake for 2 minutes. Allow phases to separate, and transfer the organic layer in a second 250-mL separatory funnel.

9.7 Extract the aqueous layer with an additional two 20-mL aliquots of chloroform as before. Combine the extracts in the 250-mL separatory funnel.

9.8 Add 20 mL of distilled water to the combined organic layers, shake for 30 seconds, and discard aqueous layer.

9.9 Transfer the organic extract into a 100-mL round bottom flask. Add 20 mL of methyl alcohol and concentrate to 5 mL on a rotating evaporator at 35°C.

9.10 Using a 9-inch Pasteur pipette, transfer the extract to a 15-mL conical screw-capped centrifuge tube. Rinse the flask, including the entire side wall, with 2-mL portions of methyl alcohol and combine with the original extract.

9.11 Carefully concentrate the extract to 0.5 mL using a gentle stream of nitrogen and a 30°C water bath, dilute to 2 mL with methyl alcohol, reconcentrate to 1 mL, and dilute to 5 mL with acetate buffer. Mix extract thoroughly. Stopper the ampule and store refrigerated if further processing will not be performed immediately.

9.12 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5-mL.

10. Liquid Chromatography (HPLC)

10.1 Table A-1 summarizes the recommended liquid chromatographic column material and operating conditions for the instrument. Included in this table are estimated retention times and sensitivities that should be achieved by this method. An example of the separation achieved by this column is shown in Figure A-1. Calibrate the system daily with a minimum of three injections of calibration standards.

10.2 Inject 25 L of the sample extract. If the peak area exceeds the linear range of the system, dilute the extract and reanalyze.

10.3 If the peak area measurement for benzidine is prevented by the presence of interferences, reduce the electrode potential to 0.7 V and reanalyze.

11. Calculations

11.1 Determine the concentration of individual compounds according to the formula:

$$\text{Concentration, } \mu g/L = \frac{(A)\ (B)\ (V_t)}{(V_i)(V_s)}$$

where A = Calibration factor for chromatographic system, in nanograms material per unit peak height.

B = Peak size in injection of sample extract, in area units

V_i = Volume of extract injected (L)

V_t = Volume of total extract (L)

V_s = Volume of water extracted (mL).

11.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

12. Accuracy and Precision

12.1 Data is not available at this time.

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APPENDIX B

INSTRUCTIONS TO ANALYST

The following instructions were sent to the analyst prior to the start of the method study.

General

Benzidine and DCB are bases and it is important to perform extractions, etc., at the proper pH. Since the compounds can be readily oxidized, especially at high pH, it is important not to allow the pH to exceed that stated in the procedure, even for brief periods of time. Once the sample is extracted it should be analyzed as quickly as possible, preferably within 8-12 hours since unknown materials in the extract may enhance the oxidative destruction of benzidine.

Since most people have not operated the HPLC electrochemical detector previously, it is important to familiarize oneself with the operation as soon as possible. Some problems which can arise with its operation are as follows:

1) Air bubbles in the flow stream must be eliminated or spiking will be noticed on the recorder. Usually this is accomplished by degassing the solvent.

2) Excessive amounts of benzidine or DCB (greater than one microgram) should not be injected since some degree of electrode coating can occur, leading to decreased electrode response.

3) All electrode connections must be properly and securely made. These connections should be made so that the operator is not likely to contact them, otherwise noise spikes will occur.

The following amounts of compound on column give approximately 1/2 scale peaks with 0.5% full scale noise:

Benzidine - 2 ng
DCB - 4 ng

Chromatographic response should be checked daily.

Treatment of Water Samples

At the prestudy conference for Category 7 it was noted that some degree of oxidation during processing occurs causing poor recoveries of benzidine, and to a lesser extent DCB. A a result of these discussions and review of the trial sample data, it appears that the source of oxidate is probably in the water, rather than any solvents or other reagents. For this reason an antioxidant, sodium thiosulfate should be added to each wastewater sample, as well as to the tap water, surface water, and distilled water samples. In order to properly maintain the pH of distilled water and tap water during processing samples must be buffered, using the procedure described later. Please note treatment with sodium hypophosphite (which was mentioned at the prestudy conference) is not effective in destroying chlorine. The exact protocols to be followed are described below. Please note that for the Category 7 study a volume of 500-mL of each water type is to be spiked with 0.5 mL of ampule concentrate.

Distilled Water and Tap Water

Add 35 mg/liter of sodium thiosulfate and stir briefly. Add 1 mL of 0.4 M sodium tribasic phosphate/liter (reagent specified in Method 605) and adjust to pH 2 with 1 M sulfuric acid. Proceed as for wastewater.

River Water

Add 35 mg/liter of sodium thiosulfate and stir briefly. Adjust to pH 2 with 1 M sulfuric acid and proceed as for wastewater.

Wastewater

These samples have been treated with sodium thiosulfate and acidified; proceed as follows. Place a magnetic stirbar in the bottle containing approximately 500 mL (±3%) of sample, add 0.5 mL of the ampule concentrate, then cap and stir 15 minutes using a magnetic stirrer. Transfer the sample to a 1-liter separatory funnel and proceed with the extraction and analysis. For the purpose of this study it is not necessary to measure the volume of wastewater; assume that it is 500 mL.

Analysts still having trouble with their reference electrodes should contact Dr. Ralph Riggin at (614) 424-5590. The problems described (loosening of the heat-shrink Teflon) occur only for recently manufactured electrodes and can be avoided by shrinking a second Teflon tube over the existing one, and are minimized by placing the electrode in 3 M sodium chloride solution when not in use.

Finally, the method states that if interferences are noted for benzidine the sample should be reinjected at 0.7V. It is likely that even greater selectivity can be achieved by reducing the potential to 0.6V. However, one must check the response for benzidine standards at the same potential, to assure that a significant decrease in response is not occurring.

APPENDIX C

TABLE C-1. RAW DATA FOR BENZIDINE ANALYSIS BY WATER TYPE LOW YOUDEN PAIR, UG/L

	DISTILLED WATER		TAP WATER		SURFACE WATER		WASTEWATER (C-74)		WASTEWATER (C-75)		WASTEWATER (C-76)	
AMPUL NO:	3	6	3	6	3	6	3	6	3	6	3	6
TRUE CONC:	1.00	1.40	1.00	1.40	1.00	1.40	1.00	1.40	1.00	1.40	1.00	1.40
LAB NUMBER												
101	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	1.60	0.00*	0.00*	0.00*	0.00*	0.00*
102	0.38	0.69	0.13	0.91	0.58	0.53	0.43	0.76	0.40	0.50	0.21	0.34
103	0.60	0.00*	0.00*	0.80	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
105	1.28	1.60	1.10	1.10	1.40	1.28	1.68	0.70	0.10	0.42	3.28*	0.00*
106	0.15	1.58	1.39	1.20	0.85	0.90	0.70	0.47	5.82*	5.00*	0.35	0.99
107	0.51	0.50	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.20	0.00*	0.00*	0.00*
109	*	*	*	*	*	*	*	*	*	*	*	*
110	0.58	0.38	1.39	0.80	0.64	0.72	1.19	0.92	*	*	1.39*	1.30*
111	1.65	0.72	*	*	*	0.43	0.75	0.53	*	0.92	0.80	*
112	0.34	0.37	0.24	3.73	0.11	*	0.18	0.19	0.48	0.71	1.09	0.63
113	0.46	1.40	0.44	1.60	0.62	1.10	0.60	0.20	0.38	0.36	0.39	0.66
115	0.63	1.10	0.77	1.20	0.69*	1.09*	0.36	1.00	0.78	1.05	0.76	*
116	0.22*	0.08*	0.13*	0.04*	0.35	0.14	0.00*	0.00*	0.58	0.00*	0.50	0.33
117	0.55	0.82	0.06	0.06	0.10	0.15	0.84	0.55	0.00*	0.51	0.09	0.04
118	1.20	1.00	1.10	1.50	0.56	0.82	0.44	0.70	0.72	0.80	0.36	0.72
123	*	12.40*	0.93	0.81	1.53	0.55	0.50	0.87	105.00*	802.00*	1.10	1.25
125	2.10	1.60	2.00	3.00	2.60*	0.40	4.40*	0.70	*	*	*	4.10*

* Rejected

APPENDIX C (Continued)

TABLE C-1. RAW DATA FOR BENZIDINE ANALYSIS BY WATER TYPE
MEDIUM YOUDEN PAIR, UG/L

	DISTILLED WATER		TAP WATER		SURFACE WATER		WASTEWATER (C-74)		WASTEWATER (C-75)		WASTEWATER (C-76)	
AMPUL NO:	1	5	1	5	1	5	1	5	1	5	1	5
TRUE CONC:	12.00	8.00	12.00	8.00	12.00	8.00	12.00	8.00	12.00	8.00	12.00	8.00
LAB NUMBER												
101	0.85*	2.00*	0.00*	0.00*	0.36*	0.00*	0.00*	0.00*	0.00*	*	0.00*	0.00*
102	9.27	5.17	8.55	5.98	9.81	4.45	0.00*	7.54	*	5.17	*	5.09
103	4.30	4.90	9.20	0.00*	8.60	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
105	10.20	7.28	9.36	6.70	9.96	6.70	6.24	3.46	5.46	4.08	6.24	0.00*
106	11.70	7.19	6.96	6.62	9.85	8.28	10.70	5.58	9.41*	7.37*	10.80	7.76
107	14.10	0.43*	11.20	5.40	2.80	1.17	0.73	0.00*	0.65	3.03	0.14*	0.00*
109	*	4.71	6.09	0.43	*	*	*	*	*	*	*	*
110	8.94	5.40	8.61	3.75	7.90	2.25	11.40	1.21	*	*	9.70*	7.50*
111	5.93	3.41	2.34	2.92	1.91	3.50	5.33	5.14	*	4.68	2.11	2.31
112	3.06	4.32	3.62	3.14	4.43	2.12	2.06	1.07	0.30	3.37	2.45	2.21
113	9.20	7.20	9.50	6.60	9.90	6.20	3.70	1.60	3.90	2.50	1.60	1.40
115	11.20	7.37	11.60	7.21	10.70*	7.10*	0.49	5.38	10.70	6.40	10.80	6.45
116	0.08*	0.09*	0.17*	0.13*	0.04	0.06	0.00*	0.00*	19.60	0.00*	0.44	0.45
117	7.63	5.30	2.93	0.35	< 0.10*	2.07	8.41	8.26	8.71	9.59	1.70	1.67
118	14.00	6.80	17.00	5.20	13.00	12.20	9.80	9.40	12.00	8.60	11.00	8.20
123	7.35	5.27	3.05	4.70	10.50	4.66	0.44	0.95	0.65	0.00*	5.77	2.50
125	5.00	8.00	11.00	8.00	6.00	5.40	11.40	7.40	10.00	*	8.10	3.10

* Rejected

< = Less than detection limit

APPENDIX C (Continued)

TABLE C-1. RAW DATA FOR BENZIDINE ANALYSIS BY WATER TYPE
HIGH YOUDEN PAIR, UG/L

	DISTILLED WATER		TAP WATER		SURFACE WATER		WASTEWATER (C-74)		WASTEWATER (C-75)		WASTEWATER (C-76)	
AMPUL NO:	4	2	4	2	4	2	4	2	4	2	4	2
TRUE CONC:	40.00	55.00	40.00	55.00	40.00	55.00	40.00	55.00	40.00	55.00	40.00	55.00
LAB NUMBER												
101	2.50*	4.60*	0.45*	4.30*	0.41*	0.00*	0.40	0.68	0.00*	0.00*	0.00*	1.60*
102	32.80	52.80	11.30	44.40	31.70	45.60	*	43.80	*	35.90	*	24.20
103	30.50	5.40	2.10	12.30	1.80	32.30	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
105	40.40	53.40	43.60	53.20	39.40	39.40	36.60	43.80	31.20	44.00	30.00	42.20
106	30.10	52.00	34.00	50.80	36.00	52.80	39.60	51.00	40.90*	52.60*	38.50	56.00
107	40.80	48.40	40.00	42.90	*	15.80	22.00	19.00	20.50	39.10	4.42*	0.48*
109	1.68	2.82	4.95	28.26	*	*	*	*	*	*	*	*
110	14.40	45.00	27.00	56.60	14.40	20.40	29.80	29.80	*	*	56.40*	50.00*
111	7.70	12.90	10.40	17.70	18.30	24.60	25.10	20.70	9.00	*	5.90	9.20
112	4.23	24.60	8.18	26.00	18.69	28.70	8.34	24.60	14.80	18.00	23.30	33.20
113	39.00	44.00	31.00	28.00	32.00	45.00	9.30	23.00	12.00	19.00	14.00	22.00
115	41.50	54.50	30.70	57.80	38.10*	52.40*	32.80	38.60	37.30	61.10	33.70	39.80
116	0.21*	0.17*	0.14*	0.16*	0.04	0.81	0.00*	0.00*	6.92	13.70	0.67	1.56
117	13.37	52.67	34.06	59.38	45.82	22.63	43.64	86.17	55.21	35.55	45.00	55.00
118	28.00	64.00	24.00	66.00	26.00	27.00	17.00	39.00	22.00	41.00	25.00	37.00
123	38.50	53.00	23.30	3.89	26.20	26.60	0.30	0.64	0.00*	645.00*	19.90	10.30
125	30.50	42.00	29.50	49.00	26.40	40.00	34.40	48.00	*	35.00	27.10	47.10

* Rejected

TABLE C-2. RAW DATA FOR 3,3'-DICHLOROBENZIDINE ANALYSIS BY WATER TYPE
LOW YOUDEN PAIR, UG/L

	DISTILLED WATER		TAP WATER		SURFACE WATER		WASTEWATER (C-74)		WASTEWATER (C-75)		WASTEWATER (C-76)	
AMPUL NO:	3	6	3	6	3	6	3	6	3	6	3	6
TRUE CONC:	1.00	1.40	1.00	1.40	1.00	1.40	1.00	1.40	1.00	1.40	1.00	1.40
LAB NUMBER												
101	1.20	1.00	1.10	0.92	0.88	0.97	5.20*	0.20	1.50	1.80	1.40	1.60
102	0.78	0.93	0.48	1.16	0.97	0.84	0.43	0.88	0.57	0.61	0.81	0.47
103	0.40	0.30*	0.10*	0.50*	0.40	1.00	0.70	2.00	0.30	2.20	3.90*	0.00*
105	1.22	1.16	0.68	1.48	2.06	1.00	2.84*	1.04	1.40*	2.10*	0.46	0.24
106	1.49	1.13	1.95	1.37	1.02	1.25	1.27	0.64	1.54	1.65	2.12*	1.27*
107	0.86	1.06	0.00*	0.58	0.00*	0.60	0.00*	0.00*	1.41	0.26	0.00*	0.00*
109	0.04*	*	*	0.42	*	*	*	0.01	*	*	*	16.07*
110	0.70	0.84	0.58	0.74	0.46	0.88	0.45	0.40	*	*	0.93	0.92
111	0.36	1.10	0.55	0.58	0.36	0.18	0.18	0.00*	0.30	0.23	0.00*	0.00*
112	0.25	0.62	0.44	0.66	0.43	0.32	0.07	0.05	0.36	0.40	0.46	0.61
113	1.90	2.30*	0.75	1.90	1.40	1.10	0.10	*	0.20	*	1.30	0.17
115	0.56	0.85	0.76	1.32	0.71	0.99	0.00*	0.85	0.78	0.95	0.76	*
116	0.77	0.94	0.50	0.89	0.67	0.27	0.00*	0.00*	0.37	0.40	0.23*	0.21*
117	0.80	0.89	0.21	0.21	0.21	0.32	0.80	0.57	1.00	1.47	0.24	0.24
118	2.40	1.30	1.50	1.70	1.00	1.00	0.62	1.10	0.72	1.30	0.62	1.00
123	0.95	10.30*	0.60	0.77	1.18	0.30	0.00*	0.00*	0.00*	0.00*	0.35	0.35
125	3.40*	3.40*	3.30*	4.00*	3.40*	1.00	2.00*	4.00*	11.00*	2.50	0.50	3.00*

* Rejected

TABLE C-2. RAW DATA FOR 3,3'-DICHLOROBENZIDINE BY WATER TYPE
MEDIUM YOUDEN PAIR, UG/L

	DISTILLED WATER		TAP WATER		SURFACE WATER		WASTEWATER (C-74)		WASTEWATER (C-75)		WASTEWATER (C-76)	
AMPUL NO:	1	5	1	5	1	5	1	5	1	5	1	5
TRUE CONC:	15.00	11.00	15.00	11.00	15.00	11.00	15.00	11.00	15.00	11.00	15.00	11.00
LAB NUMBER												
101	10.00	9.00	4.60	4.80	10.00	6.70	4.60	5.50	13.00	*	12.00	11.00
102	12.20	7.34	11.40	7.96	9.98	6.72	6.97	8.12	8.72	7.72	7.06	6.02
103	0.70	3.10	5.40*	0.40*	6.80	3.80	7.50	6.90	9.60	9.30	5.20	3.80
105	12.00	9.80	11.40	8.60	11.60	9.46	7.94	8.40	15.30*	11.20*	6.40	3.60
106	15.60	9.68	8.51	9.40	10.60	10.60	13.70	6.36	9.88	8.51	15.20*	10.10*
107	15.90	0.99	15.10	8.26	9.45	8.92	0.56*	0.00*	1.18	0.42	1.04*	1.54*
109	0.01*	4.62*	7.25	1.25	0.02*	*	0.35	*	*	0.07*	1.98	*
110	13.30	7.00	12.30	7.90	7.40	3.08	8.50	2.98	*	*	15.00	3.40
111	17.10	3.09	17.30	3.06	12.00	3.38	1.97	1.56	17.60	8.63	17.30	0.62
112	4.85	5.11	5.03	3.96	6.37	4.85	1.99	0.39	1.78	4.93	6.53	5.10
113	13.00	11.00	13.00	6.10	9.40	7.50	1.70	0.70	0.10	3.50	*	*
115	9.22	7.33	13.00	8.98	11.70	8.94	0.00*	6.12	11.00	7.73	10.30	6.90
116	6.67	5.95	4.62	4.27	0.96	1.67	2.91	4.45	0.71	2.30	3.10*	2.32*
117	9.46	6.43	7.87	4.68	8.59	2.98	10.00	9.50	12.65	15.88	7.32	3.41
118	17.00	7.60	26.00*	7.80	19.00	11.00	9.80	6.40	11.00	8.20	10.00	10.00
123	7.21	5.50	7.01	4.57	5.46	4.72	0.00*	0.00*	4.33*	0.00*	3.74	2.62
125	3.70	10.00	9.00	10.00	4.00	6.00	10.00*	10.00*	40.00*	20.00	8.00	6.00

* Rejected

TABLE C-2. RAW DATA FOR 3,3'-DICHLOROBENZIDINE ANALYSIS BY WATER TYPE
HIGH YOUDEN PAIR, UG/L

	DISTILLED WATER		TAP WATER		SURFACE WATER		WASTEWATER (C-74)		WASTEWATER (C-75)		WASTEWATER (C-76)	
AMPUL NO:	4	2	4	2	4	2	4	2	4	2	4	2
TRUE CONC:	55.00	70.00	55.00	70.00	55.00	70.00	55.00	70.00	55.00	70.00	55.00	70.00
LAB NUMBER												
101	35.00	44.00	21.00	47.00	30.00	40.00	22.00	21.00	41.00	49.00	39.00	47.00
102	39.80	51.20	35.10	46.10	39.40	53.10	*	46.50	*	51.00	*	50.10
103	42.00	0.40	5.80*	1.00*	31.70	24.70	45.00	48.10	51.30	48.70	33.40	40.10
105	54.40	62.80	35.20	59.60	58.40	47.40	48.00	48.00	50.80*	77.80*	29.40	39.00
106	50.00	38.90	47.50	38.30	49.30	53.60	41.70	56.10	39.80	42.20	50.00*	68.00*
107	49.50	53.40	53.70	57.60	*	51.60	4.87*	25.00*	39.60	34.40	17.70*	6.86*
109	15.83*	36.89*	17.55	38.05	*	0.04*	4.67	5.82	7.28*	14.03*	18.93	4.75
110	61.80	46.80	53.00	53.00	55.80	43.70	34.40	41.00	*	*	61.80	84.00
111	30.50	28.10	28.70	37.80	28.90	38.60	24.70	16.30	30.10	9.50	25.10	17.80
112	25.80	28.30	21.70	29.50	23.30	33.60	9.46	26.00	21.00	23.60	18.00	34.40
113	46.00	60.00	20.00	40.00	33.00	57.00	8.10	30.00	13.00	29.00	11.00	42.00
115	38.80	46.10	49.20	64.30	47.00	58.70	31.10	40.10	45.00	65.00	38.90	45.80
116	37.70	50.50	33.00	34.60	30.70	2.98	24.80	12.70	1.35	8.53	0.80*	16.30*
117	20.18	64.30	59.57	74.04	51.57	37.18	62.67	51.67	73.53	54.12	48.78	44.39
118	41.00	78.00	34.00	80.00	49.00	30.00	30.00	49.00	40.00	52.00	48.00	49.00
123	30.90	40.40	21.20	5.56	21.40	19.40	0.00*	0.00*	0.00*	29.10*	17.60	9.19
125	35.00	43.00	53.00	50.00	20.00	28.00	47.00*	55.00*	35.00	40.00	16.00	50.00

* Rejected

APPENDIX D

TABLE D-1. EFFECT OF WATER TYPE ON BENZIDINE ANALYSIS

** POINT ESTIMATES **

DISTILLED WATER SLOPE:GAMMA(1) = .99402

WATER	INTERCEPT(WATER-DISTILLED)	SLOPE(WATER-DISTILLED)
2	-.0856	-.0004
3	-.2850	-.0154
4	-.2167	-.0997
5	-.3963	.0862
6	-.3165	-.0130

** ANALYSIS OF VARIANCE **

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB
REG(DISTILLED)	1	907.17154	907.17154		
REG(WATER/DISTILLED)	10	12.46561	1.24656	1.78	.0622
ERROR	403	282.14432	.70011		
TOTAL	414	1201.78148			

** TABLE OF 95% CONFIDENCE INTERVALS FOR THE DIFFERENCES BETWEEN INTERCEPTS AND THE DIFFERENCES BETWEEN SLOPES **

WATER	INTERCEPT(WATER-DISTILLED) ESTIMATE	INTERCEPT(WATER-DISTILLED) INTERVAL	SLOPE(WATER-DISTILLED) ESTIMATE	SLOPE(WATER-DISTILLED) INTERVAL
2	-.0856	(-.7491 , .5778)	-.0004	(-.2469 , .2460)
3	-.2850	(-.9687 , .3987)	-.0154	(-.2685 , .2377)
4	-.2167	(-.8782 , .4448)	-.0997	(-.3478 , .1484)
5	-.3963	(-1.1354 , .3427)	.0862	(-.1895 , .3619)
6	-.3165	(-1.0306 , .3976)	-.0130	(-.2765 , .2505)

NOTE: IF ZERO IS CONTAINED WITHIN A GIVEN CONFIDENCE INTERVAL THEN THERE IS NO STATISTICAL SIGNIFICANCE BETWEEN DISTILLED WATER AND THE CORRESPONDING WASTEWATER FOR THE ASSOCIATED PARAMETER(INTERCEPT/SLOPE).

THE SLOPE AND INTERCEPT ESTIMATES FROM THIS ANALYSIS ARE NOT THE SAME AS THOSE OBTAINED FROM THE PRECISION AND ACCURACY REGRESSIONS PERFORMED EARLIER.

TABLE D-2. EFFECT OF WATER TYPE ON 3,3'-DICHLOROBENZIDINE ANALYSIS

** POINT ESTIMATES **

DISTILLED WATER SLOPE:GAMMA(1) = .93428

WATER	INTERCEPT(WATER-DISTILLED)	SLOPE(WATER-DISTILLED)
2	-.1130	.0632
3	-.2649	.0515
4	-.9544	.1710
5	-.2256	-.0027
6	-.5086	.1018

** ANALYSIS OF VARIANCE **

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB
REG(DISTILLED)	1	1239.25482	1239.25482		
REG(WATER/DISTILLED)	10	20.79161	2.07916	4.88	.0000
ERROR	465	198.21790	.42628		
TOTAL	476	1458.26433			

** TABLE OF 95% CONFIDENCE INTERVALS FOR THE DIFFERENCES BETWEEN INTERCEPTS AND THE DIFFERENCES BETWEEN SLOPES **

WATER	INTERCEPT(WATER-DISTILLED) ESTIMATE	INTERCEPT(WATER-DISTILLED) INTERVAL	SLOPE(WATER-DISTILLED) ESTIMATE	SLOPE(WATER-DISTILLED) INTERVAL
2	-.1130	(-.6004 , .3744)	.0632	(-.1060 , .2324)
3	-.2649	(-.7444 , .2147)	.0515	(-.1165 , .2194)
4	-.9544	(-1.4870 , -.4218)	.1710	(-.0111 , .3530)
5	-.2256	(-.7343 , .2831)	-.0027	(-.1802 , .1748)
6	-.5086	(-1.0404 , .0232)	.1018	(-.0795 , .2832)

NOTE: IF ZERO IS CONTAINED WITHIN A GIVEN CONFIDENCE INTERVAL THEN THERE IS NO STATISTICAL SIGNIFICANCE BETWEEN DISTILLED WATER AND THE CORRESPONDING WASTEWATER FOR THE ASSOCIATED PARAMETER(INTERCEPT/SLOPE).

THE SLOPE AND INTERCEPT ESTIMATES FROM THIS ANALYSIS ARE NOT THE SAME AS THOSE OBTAINED FROM THE PRECISION AND ACCURACY REGRESSIONS PERFORMED EARLIER.